

nature

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Room at the top — for whom?

REPORTS on the state of British industry, its use of qualified scientists and engineers, the attitude of young people towards an industrial career and recipes for supposed improvements are now descending on the office in such profusion that we can no longer guarantee even to collect them from our harassed reception before dispatching them, bundled in their thousands, to a company warehouse somewhere outside Basingstoke, there to be pulped. Two recent arrivals, however, somehow crept under the door and so have at least been afforded a cursory glance by our jaded editorial staff. They are 'Education, engineers and manufacturing industry', a report to the British Association for the Advancement of Science by a small investigating team, and 'University-industry relations', the government's reply to some recommendations made by the Common's Select Committee about a year ago.

At that time, we were critical of several of these recommendations, which seemed rather poorly worked out, at least in the case of the proposed revival of the concept of SISTERS, Special Institutions for Scientific and Technological Education and Research, a step in the wrong direction of isolating science and technology from the broader world. The government's response is equally unenthusiastic, indeed its paper as a whole reads like a long succession of "we don't think this is a good idea", "we think this is a good idea and it is already happening", "we think this is a good idea but someone else should do something about it". The someone else is, of course, industry.

But one recommendation commended to industry is not so easily shrugged off. The Select Committee had said that industry should do something about offering attractive salaries and moving qualified personnel in to senior management with the ease that it is done in Germany, France and the United States or by their British contemporaries who are lawyers or accountants. A good generalised knockabout, but the British Association report puts it in much clearer perspective. It is fairly widely known that on an international comparison, British median salaries are low at the shop floor level and get relatively lower the more responsibility is

assumed. A new survey, released in part to the British Association, looks at the way that even these inferior salaries discriminate against the scientist and engineer in productive industry. Median figures (for October 1975) for about 22,000 professionals show that at all ages accountants and lawyers get paid the most, engineers and scientists the least. Even at 25 the accountants and lawyers are £1,000 ahead; by 40 their median is £9,500, that of arts graduates £8,500, that of scientists £7,500 whilst engineers are commanding a mere £6,600.

Maybe the average non-scientist in industry really is much smarter and earns his or her differential, but no-one yet seems to have said so explicitly. More likely the growing differential reflects the expanding opportunities open to the non-scientists as time goes on. More opportunities mean more rounded employees, soon to be fit for even more opportunities. Less opportunities mean more type-casting as narrow minded characters fit only for the backroom and the sales department.

The matter is not so easily laid only at industry's door, however. The government itself employs tens of thousands of qualified scientists and engineers. Their upward path until the age of 40 is fairly well-defined and somewhat better paid than in industry. But thereafter, stagnation. Ten years ago the Fulton report on the Civil Service, recognising the problem, proposed an 'open' structure at the top of the service to encourage some cross movement among real high fliers. In practice this has meant little. Certainly laboratory directors are at least nominally in the open structure, but when one asks about other scientists and engineers who have made the move into more general high level management, the same name or two keep cropping up. Whether the waste of intelligent human resources would be ameliorated by extending the open structure further down the service or by making it easier for the frustrated to retire earlier, who knows? But at the moment the government is in no strong position to commend industry to take more scientists and engineers into senior management when it shows so little inclination to do so itself. □



Refusnik scientists: keeping science alive

Max Gottesman, from the Laboratory of Molecular Biology at the National Cancer Institute at NIH, was one of ten Western scientists who attended the special four-day Jubilee meeting of the refusnik Sunday Seminar in Moscow earlier this year; the seminar dissolved itself for a 'summer recess' in May following official pressure, and reconvened again last Sunday under Viktor Brailovskii. Below, his views of the refusniks' position

FIVE years ago, the first of the refusnik Sunday Seminars on Collective Phenomena met in a Moscow apartment. Refusniks, unlike dissidents who wish to change Soviet Society, are Jews who wish to leave Russia, usually for Israel. In the case of refusnik scientists, visas to emigrate are often refused on the grounds that the applicant is in possession of "classified information". Not only is their emigration forbidden, but their official scientific life is terminated. They are expelled from their laboratories and denied library privileges; even their telephones are removed. The cut-off is usually very effective: none of Professor Benjamin Levich's colleagues, for example, nor any of the students he had trained over the years, spoke to him after he applied to emigrate.

In an attempt to stay alive scientifically, the refusniks meet together in someone's apartment each Sunday afternoon to discuss science. These meetings have come to attract Western scientists invited to Soviet-sponsored conferences, and they present papers; sometimes establishment Soviet scientists discretely listen to the presentations while remaining in the kitchen. But the special Jubilee meeting held to mark these five years was also accompanied by the constant presence of KGB men stationed outside Dr Mark Azbel's apartment, where the seminar was held.

They followed the Western scientists wherever we went. Usually consisting of four men in a taxi, they made no attempt to disguise the fact that we were being followed; they were there clearly to intimidate us. One afternoon, a truckload of soldiers unloaded below Azbel's window and lounged around several hours before leaving. A parked van, left with the motor running and no driver, contained the monitoring devices used by the KGB to follow the conversations in the apartment. Between the sessions, however, matters of a non-scientific nature were discussed, and from these talks and exchanges of written notes I obtained a picture of the present status of the refusnik scientists.

How the refusniks manage to survive varies from case to case. Some had accumulated savings before applying for an exit visa. Of course a long five- or six-year period of refusal cannot be prepared for in this way. During refusal, they find occasional work, sometimes as manual labourers and sometimes as tutors. Unfortunately tutoring positions tend to be transitory; a discrete call from the KGB to a family employing a refusnik tutor usually has the expected effect.

It is clear that the scientists who have entered into refusal have a common motivation: to escape the severe and worsening anti-semitism in Russia. The Russians wish to exclude Jews from the sciences and other professions. At the present time, it is difficult or impossible for a Jewish student to enter a first-rate university. An example: the medical school in Odessa, a city with a large Jewish population, has not a single Jewish student. Thus many Jews are driven to emigrate to assure their children equal educational and employment opportunities. Current Soviet anti-semitism comes after a long period of relative tolerance. A generation of Jews has been assimilated, and the refusniks find themselves without an inheritance of Jewish culture.

They are anxious to regain their heritage—and to learn Hebrew—in the face of severe pressure from the authorities. It is no accident that the two refusniks currently in prison, Joseph Begu and Anatolii Shcharanskii, were the most active in disseminating Jewish culture. And yet, in spite of the threats, there were several refusniks at the seminar I attended wearing Israeli pins in their lapels.

Why don't the Soviets just let the refusniks emigrate? In fact, some are permitted to go; but the rate is slow, and getting slower. The Soviets fear that an easy emigration policy will result in Jews as well as other national groups demanding, *en masse*, to emigrate, thus threatening the stability of the State. Also, the Jewish scientists have a special problem. The justification of the Soviet system is 'scientific'; Marxist-Leninist analysis of history and economics has proved, in a manner as certain as $E = mc^2$, that Soviet society is structured correctly. For a scientist to desire to emigrate is unthinkable. It should be mentioned that the only valid reason for leaving Russia is to rejoin one's family abroad.

What can be done? In view of the fact that the Soviets greatly value scientific contacts with the West, certain courses of action are feasible. In particular, international meetings and exchanges can be used to help the refusniks. Refusnik scientists wish to participate in these open meetings and could be invited. If such participation is denied by the authorities, Western organisers should consider either the possibility of moving the meeting from Soviet territory, or of limiting Soviet participation in meetings held in the West. In the case of the International Genetics Conference, to be held in Moscow in the summer of 1978, a precedent has been set. A prior conference was moved from the United States to Canada in the 1950s when the US State Department refused to issue visas to certain foreign scientists.

Publicity is essential to keep the refusniks from simply disappearing. Official Soviet visitors should be reminded that the treatment of refusnik scientists threatens Soviet-Western exchange programmes. Since visitors are invariably debriefed by the KGB on their return, this is an effective means of reaching the Soviet authorities. It should also be remembered that the critical decision as to whether a refusnik's work is 'classified' is made by his departmental superiors, or the chairman of his department. These are often the very scientists who are sent to Western conferences and exchange programmes.

Finally, it should be stressed that scientists visiting Russia are warmly welcomed at the Sunday Seminar, and are doing a great service to the refusniks by attending. For addresses and other information contact the Committee for Concerned Scientists, 9 East 40 Street, New York, New York, 10016. □



Sunday Seminar scientists in November 1976

Tethering the tide

T. L. Shaw examines the prospects for harnessing tidal energy in the Severn Estuary

FACED with a possible world fuel shortage, the UK Department of Energy (DEN) is taking stock of domestic energy sources. Together with industry, it has already provided money for scientific and technical studies on the contribution four 'renewable' sources—wave, solar, geothermal and wind—could make to meeting future demands. It is now looking at the part tidal power could play.

The DEN's recently-released Energy Paper number 23, which discusses three commissioned reports on harnessing tidal power in the Severn Estuary, concludes that although tidal schemes are technically feasible, cost and the long time estimated for construction may rule them out. Nevertheless, studies to identify one or more schemes for wider appraisal are now being seriously considered.

The tides have the particular advantage of far greater energy density than the other renewable energy sources. In the Severn Estuary, for example, tidal energy is thirty times denser than power derived from average waves on the UK's Atlantic seaboard. Individual tidal schemes could produce many thousands of megawatts of electricity but, for maximum benefit, they would have to be integrated efficiently with other (essentially thermal) generating plant.

This enormous potential could be harnessed by building a dam to separate part of the coastal inlet from the sea. Turbines that might resemble conventional hydro-electric propeller machines would be set in fully sub-

merged tubes passing through the barrage. A difference in water level either way across the barrage (during flood and ebb tide) would create flow through these tubes and so an electrical output from the slowly rotating turbines.

In the Severn Estuary, the UK has perhaps the best site in the world for producing electricity from tidal power. At least 10% and as much as 20% of the UK's present electrical needs could be met from a barrage either within the estuary (10%) or seaward in the Bristol Channel (20%). These percentages are comparable with those currently supplied by nuclear installations and oil and gas-fired stations respectively.

But this is not simply a case of one electrical source competing against another. Each has characteristics which influence its most efficient hence preferred use: nuclear stations, for example, should run continuously, whereas gas turbines are best run in short bursts at times of high demand. The output of aerogenerators is fairly regular over a period of hours and maybe days while that of wave generators may be consistent on an even longer scale. Tidal output suffers from being intermittent but it is accurately predictable. There is no reason, however, why each source, provided that it can be commercially developed with respect to its operating value, should not contribute to the total generating network.

Recent studies

Last year the DEN commissioned two investigations into aspects of the Severn barrage. The questions the investigations set out to answer were: is construction and closure of a barrage

technically feasible? how long would it take to build? what would be the likely cost? and what would be the effect of the barrage and its operation on tidal range? They were issued respectively to the Netherlands Engineering Consultants Foundation (NEDECO), which has great knowledge in maritime affairs especially coastal protection, and to the UK Department of the Environment's Hydraulics Research Station (HRS), which, since it was established some 30 years ago, has worked extensively on coastal hydraulics.

For their studies both NEDECO and HRS chose to consider barrages in positions between Cardiff and Weston-super-Mare, where 10% of the UK's present electrical demand could be produced. Most attention was paid to proposals by Professor Eric Wilson of Salford University (the simple single-basin, tide-related scheme) and myself (the more complex two-basin arrangement providing storage and giving power by day as required. Summaries of each study are as follows.

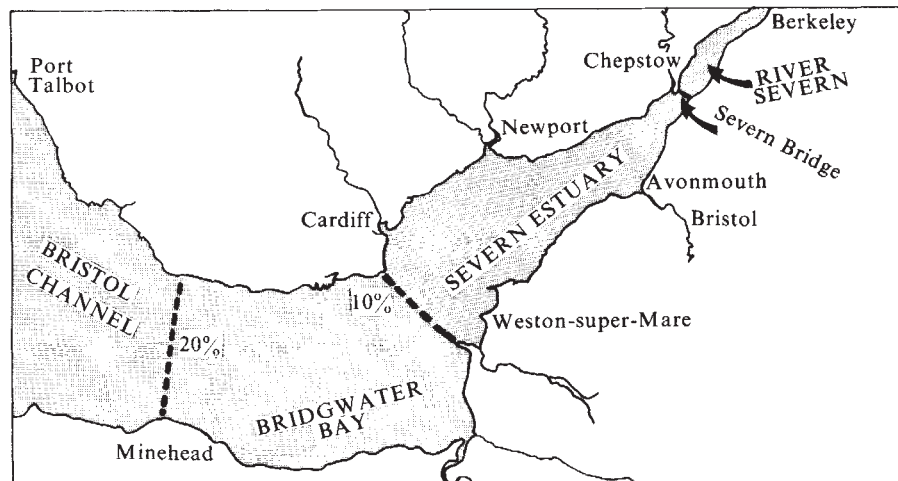
NEDECO

In the absence of adequate field data, NEDECO was obliged to make a number of assumptions about hydrodynamic conditions at the chosen barrage site, in particular data describing wave climate. The serious lack of adequate geological and sedimentological information was met by surveys carried out by the UK Institute of Geological Sciences. It identified exposed limestones and marls over a much greater proportion of this part of the estuary than the previous scant scientific data had suggested. Localised fine deposits, mainly sand and shingle are thought to be shallow, implying a firm foundation throughout for a barrage.

Based on "the impressive experience obtained in the planning and execution of the Delta Works (in Holland) together with the immense increase in know-how and expertise of recent years in the offshore industry," NEDECO is optimistic that civil engineering aspects of the barrage could be carried out satisfactorily, though it appears to have underestimated the magnitude of the challenge successfully met by British industry of placing concrete caissons in 150 m of water in the remote and exposed North Sea, compared with the task of siting correspondingly much smaller units in no more than 30 m of sheltered water in the Severn Estuary.

But the siting of caissons for the barrage raises some problems not met in the North Sea, in particular the strong currents that accompany the

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high tides. The positioning and sinking of caissons in currents exceeding 3 metres per second could prove hazardous, but given proper planning they could be placed during slack water 'windows' which occur frequently and predictably.

Doubts also exist on the accuracy of NEDECO's estimates of extreme wave heights at the chosen barrage site. Unfortunately those local measurements that have been made are for such short periods that the validity of statistical extrapolation is questionable. Another factor is the apparently sheltered position of the barrage compared with that of the one long-established wave recorder 100 km seawards. In the absence of other evidence, however, NEDECO's estimates cannot yet be dismissed.

Extra cost, NEDECO claims, will have to be incurred in building offshore-dykes to protect the turbines in the single-basin barrage from direct wave attack. If a two-basin scheme is adopted, however, the problem need not arise: the second basin would essentially serve the purpose of offshore dykes.

NEDECO estimates that the civil engineering task alone for the two-basin scheme will require four years of preliminary study and 16 years to build. This could be taken as sufficient to rule out the scheme on economic grounds. But it does agree that the timescale could be shortened, though with an increase in capital cost. By contrast, the UK construction industry feels that a timescale of about eight years, similar to that of most thermal plant, is realistic.

An alternative to help save on interest charges, would be to secure some energy from the scheme long before completion, increasing it as the scheme advances. The full output capacity of the scheme then would not be suddenly available to the network, and would permit efficient integration. Hence 16 years may not be unreasonable, though the various electrical merits of phased construction of a barrage are not yet clear; studies well advanced at the University of Bristol, funded by the Science Research Council, are exploring the possibilities. The concept of a two-basin barrage scheme developed first to operate in the 'single-basin' mode, as discussed earlier, may make good sense. NEDECO's study did not consider phased integration.

Hydraulics research station

The general lack of field data also made it difficult for HRS to define its problems, especially the seaward boundary conditions for its study's two-dimensional mathematical model.

Coastal and offshore tide modelling has moved a long way forward in the past five years, since it was recognised that local boundary changes can have effects at a considerable distance depending on their extent and the local tidal behaviour. Although earlier models, including that used by NEDECO, assumed that conditions at the seaward end of the Bristol Channel would not be unchanged by a barrage, HRS included the Celtic and Irish Seas in its model.

It is perhaps unfortunate that the 'large area' model of HRS predicts that the barrage will marginally amplify the tides whereas 'small area' models suggest the opposite. The right answer must be found, not least because, as matters now stand, the accuracy of both is under question.

But these doubts hide two aspects of the work of HRS. First, that it is something of a pioneering exercise in its own right and deserves recognition as such even if deficiencies later emerge. Secondly, an increase or decrease of tidal amplitude by up to 1 metre or so is sufficiently small in real terms to conclude at this stage that a barrage operating in the region Cardiff-Weston will not destroy the natural energy it is designed to harness. Although it must be a matter of some urgency to resolve why different mathematical models give different answers, and in particular to produce a model giving the right answers, it would only be wasteful to repeat these studies without knowing where the barrage might be sited, and understanding pump and turbine operation and induced oscillations (seiches) in the enclosed basin(s).

When reporting 'The situation in 1975', Energy Paper number 23 quotes that the Secretary of State's Advisory Council on Research and Development for Fuel and Power (ACORD) and the DEN came to the opinion that "in view of the uncertainties surrounding some of the aspects of the studies (of the barrage, as previously carried out by others), a more vigorous examination should be undertaken of certain key technical questions in order to clarify the situation further".

Of the three questions posed, the work of NEDECO and HRS should have answered two to the satisfaction of ACORD and the DEN. I hope that the third, namely "what would be the environmental consequences, and could they be quantified in cost terms?", will also be answered in similarly sufficient detail by the book *An Environmental Appraisal of the Severn Barrage* (second edition).

The next step is aptly stated by NEDECO: "The chance exists that we could contribute towards the

● The future for tidal energy in Britain has at last been clarified. There have been strong calls for further studies by the House of Commons Select Committee on Science and Technology in its fourth report published last week and at the Secretary of State for Energy's 'Forum on the Severn Barrage', held in Bristol the week before.

Although the potential of the tides and the consequences of a barrage are fairly well understood, gaps in knowledge remain. The Select Committee, which has come out in support of tidal energy ahead of the other renewable sources, proposes that an independent Severn Barrage Committee, composed of technical, financial and administrative experts, be set up to assess Severn Barrage schemes and deliver a studied opinion to the Secretary of State. It highlights the unresolved problems of choosing a barrage location and method of operation and suggests that other studies should also be done. Outline costings, provisional construction methods and timescales should be sought by a "fairly early date . . . to command general agreement".

The Secretary of State's 'Forum' provided the DEN with first-hand evidence on most of the many relevant topics, for example energy cost/benefit estimates, environmental consequences and the widespread employment, industrial, commercial and recreational merits of the project. Though opposition was scarcely evident, many speakers were cautious because so much remains unclear. Summing up Mr Benn, the Secretary of State, said that all relevant interests must be represented in the next important phase of project definition and that a staged approach allowing for reappraisal of points was essential. (The case for a pilot installation has wide support.)

While the DEN may not altogether deserve the Select Committee's criticism of being "excessively timid" about tidal energy, its scale of research funding must increase 100-fold if these unified calls for action are to be answered. The national benefits at stake ought to make it easy for a cabinet to persuade the government to provide the still-modest sum now needed.

creation of a very clean type of energy production, the value of which can only increase because of its increasing scarcity on a worldwide scale". British industry is ready to contribute towards these investigations.

The capital cost estimate of up to £4,000 million quoted in Energy Paper number 23 might imply an expensive power station, but current unit costs for other plant tend to refute this. However, simple comparisons are dangerous when like objects are not being equated. It is much better that the next phase of study of the barrage should be carried out to identify its real electrical (and other) benefits before economic judgments are reached. A comprehensive but objective programme for the next 18 months is needed. □

OTS

Second chance

Last week, Europe's Orbital Test Satellite was destroyed shortly after launch. Judy Redfearn reports

EUROPE'S hope for a sophisticated system of communications satellites by the early 1980s might well have gone up in a cloud of smoke with the European Space Agency's (ESA) Orbital Test Satellite (OTS) last week, had it not been for ESA's foresight. In the wake of two mishaps by NASA earlier this year—its failure to launch Geos into the correct geostationary orbit and an accident with a Delta launcher which meant that OTS could not be launched in June as planned—ESA made sure that it went ahead with a plan to take out insurance cover for the launch of OTS and the integration of a second flight model. The insurance, to the tune of \$29 million at a premium of about \$2 million, was placed by Bowring of London throughout all the countries taking part in the project in proportion to their financial commitment to it.

As fate would have it, ESA's precaution was extremely wise. Seven miles above Cape Canaveral's main launch pad and only 54 s after a seemingly perfect launch, the Delta 3914 carrying OTS began to disintegrate and exploded just prior to a signal of destruction. The insurance cover, however, gives Europe

another chance. As with all satellite missions, ESA built a second OTS identical to the first which could be ready for launch by next March. This means that the OTS test and experimental programme could still be compressed to be completed within the original time.

A second chance is particularly important for the OTS because, unlike Geos, it has considerable commercial potential. It is Europe's first communications satellite and is the forerunner of four similar satellites which are planned for launch by Ariane, Europe's own launcher which is still being developed, between 1981 and 1990. They will carry a large part of intra-European telephone, telegraph and telex traffic and will relay European television programmes. The OTS's task is to test much of the equipment which will be used on the operational satellites and to provide a pre-operational European traffic capacity.

The decision on whether or not to go ahead with the second OTS launch early next year will probably be taken by ESA officials this week. As the only launcher available to do the job is the American Delta 3914, they are probably hoping for some assurance from the Americans that a similar accident is extremely unlikely to happen again. Although the success rate of Delta launchers is very good—134 have been launched since 1966, 122 of them

successfully—the history of the Delta 3914 is not so happy. Three of them have been launched and the OTS marks the first failure: but the launcher which was damaged in May this year and caused a three-month delay to the OTS programme was also a 3914. In that case, one of the booster rockets fell off the main Delta vehicle because of a defective bolt.

Late last week, NASA set up a seven member team of inquiry to look into the incident. It hopes to find out whether or not the fault lay in the basic Delta vehicle or the attached rockets before 13 October, the planned launch date of the International Sun-Earth Explorer (ISEE) in which ESA has a large interest. If the fault is found in the Delta 3914's strap-on rockets then it should be safe for the ISEE to go ahead as planned as the Delta 2914, on which it will be launched, uses a different rocket configuration.

Until late 1980 when the Ariane launcher is ready for use, ESA will have to launch its satellites on US vehicles. By the early 1980s, however, there may be another option if a West German company, Orbital Transport- und Raketen-Aktiengesellschaft (OTRAG), is successful in developing its own heavy launch vehicle. So far, it has spent \$30 million on the project and hopes by 1981 to be launching satellites from Zaire. ESA will not discuss the possibility of using OTRAG launchers until it knows whether they, and Ariane, will work. □

BRITAIN

Timely delay

The Council for Science and Society has recently published a report called The Acceptability of Risks. Alastair Hay reports

IN spite of a 12-month delay, the Council for Science and Society's report, *The Acceptability of Risks** published last week has arrived at an opportune moment: the explosion at Flixborough, and the release of tetrachlorodibenzo-p-dioxin at Séveso are recent memories, and the public enquiry at Windscale is still in progress. Its findings and recommendations will certainly provoke heated debate.

Risks, it claims, are very unevenly distributed; those in the UK at greatest risk are "concentrated in the homes,

communities and workplaces of manual workers and their families". In a harsh message, the report states that positive action to remove this inequality is urgently needed, and that it regrets that "hazards of all sorts have been shamefully neglected in scientific and technological research".

The report also points out the potential danger of regulatory agencies being weakened and rendered ineffective by financial constraints and inappropriate standards. Indiscriminate use of the terms 'safe' and 'acceptable' are to be avoided, it says, as both are ambiguous and subject to a wide range of individual interpretation.

Appendices deal with specific issues such as nuclear radiation, ammunition and explosives, the hazards of asbestos, and the Flixborough Court of Enquiry. In a disturbing commentary on the conduct of the Flixborough court investigation, one of the authors of the

report, Brigadier R. L. Allen, claims that the enquiry was too narrow; that some relevant evidence was not considered; and that statements by the court to the effect that "Nypro [owners of the Flixborough plant] were safety conscious" were inappropriate in the light of the evidence presented. He says that this is illustrated by Nypro's breach of licensing arrangements when it stored 43 times more inflammable fluid on site than it was entitled to do; a fact which severely hindered rescue operations after the initial explosion.

In conclusion, the report recommends areas of investigation to be considered by working groups and which could provide useful information for 'risk management' in the future. Also suggested is the possibility of the appointment of 'risk advisers' in particular occupations and localities.

A few of the abuses the report refers to have been dealt with during its delay at the printers. Few will consider this more than a minor fault. □

**The Acceptability of Risk* is available from Barry Rose (Publishers) Limited, Little London, Chichester, Sussex, £5.00.

USA

Battling against the inevitable

Years of hitherto unsuccessful effort in the US Congress have culminated in a bill providing for an increase in research on earthquakes. Chris Sherwell reports from Washington

DEVELOPMENTS now reaching a climax in Washington may not do for earthquake research what previous much-publicised efforts did for space and cancer research, but the comparison may not be entirely inappropriate. The fanfares may be absent and the vistas uncertain, but an agreed commitment in principle which also has legislative form now promises a long-awaited boost for work in an exciting area and marks an important move from disaster relief to disaster prevention.

The latest step along the tortuous path to a comprehensive coordinated programme to reduce the hazards posed by earthquakes came last week when the House of Representatives approved by a margin of 229 votes to 125 an authorisation bill on which two House committees had reported. The bill was due to go back to the Senate late last week for consideration of the minor differences from the Senate version

passed earlier this year, and was expected to find its way to President Carter's desk for signature shortly thereafter.

The bill, which when enacted will be known as the Earthquake Hazards Reduction Act of 1977, is designed to reduce the risks to life and property of future earthquakes in the United States. The logic underlying it is simple: earthquakes are inevitable, and population increases and urbanisation enhance the dangers they pose. To minimise both the risks and potential losses, a coordinated programme to increase research and to help apply its findings and generally improve the readiness of vulnerable areas for emergencies is necessary. The bill creates a \$215-million three-year federal programme to do just that.

Though expenditure is scheduled to begin at the start of the coming financial year (fiscal year 1978) beginning on 1 October, the money must still be appropriated in a separate process. In the meantime the agencies slated to use it must rely on appropriations already granted them for that period. The two major agencies involved—the National Science Foundation (NSF) and the US Geological

Survey (USGS)—expect to feel the improvement over recent years quite soon, however, because the appropriations already granted are based largely on the same formulation used in drawing up the bill.

That formulation was contained in a report of a joint advisory committee chaired by Dr Nathan Newmark and published a year ago. Entitled *Earthquake Prediction and Hazard Mitigation: Options for USGS and NSF programs*, the report outlined low, intermediate and high levels of support for earthquake research, all of which were higher than funding levels in past years. The intermediate level, which President Ford used in his proposed budget for fiscal year 1978 and which President Carter retained in his revisions, provided for expenditure of nearly \$54 million, an increase from \$22½ million the previous year. This was split approximately 52 : 48 between NSF and USGS (in favour of NSF). Under the bill passed by the House, some \$55 million would be split 50 : 50.

Going by the breakdowns given in the Newmark Report, the two agencies would each spend about 10% of their 1978 allocations on fundamental studies of the causes and mechanisms of earthquakes. NSF does not expect to see any great difference in this area, but on the engineering front, which

No long encounter with Halley's Comet

THE National Aeronautics and Space Administration (NASA) has reluctantly abandoned an ambitious and expensive plan to conduct a close-range study of Halley's Comet as it swings around the Sun in 1986. The plan would have entailed launching a spacecraft in 1982 on a complicated journey, culminating in an extended rendezvous with the comet three and a half years later.

According to NASA officials, development of the spacecraft together with a new propulsion system to manoeuvre it into Halley's orbit would have cost \$500–600 million. In particular, considerable outlays would have been needed in the next two years to make the 1982 launch date, and NASA simply doesn't have enough money in its budget.

Consequently, the agency has been forced to drop the idea of paying an extensive visit to Halley's Comet, and it is now looking into less expensive plans to launch a spacecraft to make a brief encounter with the comet soon after it emerges from behind the Sun. In addition, NASA is con-

sidering the possibility of developing a spacecraft to make a lengthy visit to the comet Encke in 1987.

The decision to forego the chance of a long encounter with Halley's Comet will be a major disappointment to many space scientists. Comets have recently come under serious study because they may provide some clues to the origins of the universe. According to one widely-held theory, they are like giant dusty snowballs, made of condensed matter from outside the solar system. As they swing past the sun, heat vaporises material from the comet's nucleus, which then forms the so-called tail.

A trip to Halley's Comet, culminating in an extensive visit, would have been a difficult proposition, however. The chief problem is that the comet orbits the Sun every 76 years in the opposite direction from the Earth, and a complicated series of manoeuvres would be required to bring a spacecraft to the same path and speed as the comet.

For the past year, NASA has been looking into two propulsion systems

which could provide the required manoeuvres. The first, a so-called solar sail, would have consisted of an immense plastic sheet, deployed from the spacecraft on booms, which would act like a sail in the solar photon stream. The second idea, an ion drive system, would involve the ionisation of mercury vapour, which would then stream out of the back of the spacecraft to provide thrust. Last week, NASA announced that it has chosen the ion drive system which will be developed for a possible mission to visit Encke in 1987 and which will also be used to power future interplanetary probes.

Though the plan to rendezvous with Halley's comet has been abandoned, NASA may still launch a spacecraft in 1985 to fly close to the comet and pass through its tail. Such a mission would not require a special propulsion system because complicated manoeuvres will not be required to match orbits. It would consequently be much cheaper, but the scientific benefits would also be much less.

Colin Norman

would take some 60% of the NSF allocation, research funding will almost double in the coming year. This effort will focus on the design, planning, construction and use of various man-made works to resist earthquakes. Another 20% of NSF funds would probably go to related socio-economic and policy areas.

On the same basis, USGS is likely to devote about half its allocation to the development of methods to predict the time, place and magnitude of future earthquakes. Another 35% or so would go on assessing earthquake hazards, while a small amount would be spent on studies of artificial earthquake induction.

As for fiscal years 1979 and 1980, the 50:50 split between the two agencies would continue for budgets totalling \$70 million and \$80 million; the breakdown could be expected to follow broadly the same pattern. If the patterns of spending are predictable, however, the scientific results are not. Recent scientific progress in the field has probably assisted the bill's passage; reliable earthquake prediction seems more likely than ever before.

Of equal importance for the bill, however—apart from an unsubstantiated sense that more earthquakes than usual have struck in recent years—was the support given by the Carter Administration, notably by the Office of Science and Technology Policy in the White House, which is headed by the President's science adviser, Frank Press, himself a geophysicist. Indeed, the bill reflects many of the Administration's preferences. When it came out of the House Interior and Insular Affairs Committee, under which USGS falls, the important difference from the bill it received from the House Science and Technology Committee, and which put it in line with the Senate version, was that it contained no specific institutional proposals. The bill it received had suggested an Office of Earthquakes Hazards Reduction, a National Advisory Committee, and an Earthquake Prediction Evaluation Board.

Given the President's desire for flexibility while pursuing plans for government re-organisation, these suggestions were premature. Accommodatingly, the bill thus provides simply for designation of a "lead agency", and the usual establishment by the President of roles and goals.

On Capitol Hill not all reactions

Controlling technology flow

QUESTION: how does a technologically and militarily sophisticated superpower, which is committed to free trade principles, contain the contribution that its much sought-after exports might make to the military capability of potential adversaries? **Answer:** it's a problem. The latest round in a recurring debate in the United States on the matter came immediately before the Labor Day weekend at the end of August, when the Secretary for Defense, Harold Brown suddenly released a five-page memorandum sent to various officials in defence-related agencies.

Cast as an interim policy statement on controls over the export of US technology, the memorandum draws on the recommendations from the Defense Science Board Task Force, published last year as the so-called Bucy Report (see *Nature*, 15 April 1976). This indicated that controls over the flow of strategic or "critical" technology to Communist countries had broken down in recent years, and suggested that the United States should refine the list of relevant technologies and apply sanctions where they were unwarrantedly passed on, directly or otherwise.

The "interim internal guidance" which the Brown memorandum provides for the Department of Defense (DOD) covers exports both to allies and to potential adversaries. It says DOD will support the transfer of critical technology to countries with which the US has a major security interest where this can strengthen collective security, contribute to NATO standardisation and enhance the return on R&D. But with both allies and other non-Communist countries, DOD will also assess the recipient's "intent and ability to prevent either the compromise or the unauthorised re-export of that technology", relying on the intelligence and security communities to help discover any breaches. Violations would result in sanctions.

Regarding exports to potential adversaries, a "presumption for recommending disapproval" will operate where these involve a revolutionary advance in defence-related technology. But where they involve end

products not of strategic importance or with virtually unextractable valuable technology, DOD will normally recommend approval. Either way, the key consideration would be the recipient's military capability.

According to the memorandum, the Department of Defense will be asking the Commerce Department to change present regulations so that exports of critical technology to all countries would require a valid licence; DOD will also recommend a streamlining of application procedures to minimise delays. In addition, DOD will suggest that the State Department negotiate new measures to control the flow of technology to Communist countries with the Consultative Group Coordinating Committee (COCOM), which is a group of NATO countries and Japan.

The primary objective in all this, of course, is to protect US lead times in the application of technology to military capabilities. The hope is that this will be more readily achieved through an emphasis on technology rather than end products, and by having the policy cover all countries using faster procedures. The provision allowing for DOD "recommendation" will help here; so too will the plan to maintain a continuously updated list of critical technologies. No indication was available, however, regarding the timetable for a fully national policy; that will bring in other government departments.

In the meantime, one minor worry remains. A covering letter to the Bucy Report described, as potentially an "area of concern", the scientific exchange agreements under which scientists move between the USA and the Soviet Union. The Brown memorandum does not take this matter up in any real detail, but says that when the potential for "inadvertent transfer" is high, DOD will recommend restrictions "on the amount, extent or kind of interpersonal exchange". Such exchanges are already subject to certain procedures and regulations, however, and the Head of the Exchanges Office at the State Department says the memorandum implies no changes.

Chris Sherwell

Colin Norman leaves Nature this week to go to the Worldwatch Institute in Washington. He has been our Washington correspondent for about six years, having previously worked in the London office. With his departure the journal loses a distinguished observer of the scientific scene.

were positive. Senator Alan Cranston of California, who has sought earthquake legislation for five years, naturally welcomed passage of the bill as a "historic step"; last year he saw his own bill, already passed by the Senate, die in the House at the end of

the session. But in the House a Maryland Republican was more caustic: a Congress which could not detect an inflationary impact in spending a quarter of a billion dollars, he suggested, "could not possibly detect an earthquake". □

PAKISTAN

Voice for science

Academics and industrialists have been discussing the role of R&D in Pakistan. Azim Kidwai reports

PAKISTAN has remained in a state of political flux since the general election last March when Mr Zulfikar Ali Bhutto's Peoples' Party won a doubtful victory. The army takeover and the introduction of martial law in early July has also had its sobering effects; but in the face of this and the prospect of another, perhaps emotional, election next October, Pakistan's R&D effort seems unusually resilient.

This is reflected in the fact that the consensus reached by important representatives from education, science, technology and industry at the National Seminar on Cooperation between Universities, Research Institutes and Industry, recently held in Karachi, may have significant effects on the promotion of R&D in Pakistan. The seminar was unique in that it was organised by the Federation of Pakistan Chambers of Commerce and Industry and was attended, among others, by three vice-chancellors, the Chairman of the Pakistan Council of Scientific and Industrial Research, the Secretary of the Ministry of Science, several heads of research institutes and many leading industrialists. It was, in fact, a sequel to a UNIDO Adhoc Expert Group meeting on the same subject, which was held in Vienna last December and in which Pakistan participated.

The Karachi moot, however, reflected new trends in Pakistan one of which was that the private sector of industry, which had hitherto been largely apathetic towards scientific and industrial research, had started showing awareness of its importance. A fruitful outcome of the three-day deliberations was the formation of a National Research Utilisation Board by the Chambers of Commerce and Industry, consisting of representatives from universities, research institutes and industry itself. It is to promote industries based on indigenous research.

The main bottleneck so far in making use of research has been the absence of any proper mechanism to rope industry into the R&D effort. The entrepreneurs in industry have largely settled for 'turn-key' jobs on proven products and for technology developed by foreign firms. The element of challenge and the calculated gamble in using products and processes developed locally has rarely been acceptable to

the industrialist.

The position now appears to have changed considerably with the tight balance of payments making it imperative to exploit indigenous raw materials and local technological talent. The dearth of foreign exchange has had its repercussions on the industrial sector. For the first time, industrialists are coming round to the view that the limited resources of the country call for an organised approach based on self-reliance and for the mobilisation of the meagre R&D resources. The technology which will be applied, however, will need to be appropriate to the physical, human and capital resources available.

Until now, there have been two weak points in the structure. The universities have been dominated by academics and have contributed almost nothing to applied research and the development of industry; and there has been a lack of interest on the part of industry in the government's R&D effort. The recommendations which came out of the seminar are for more collaboration between industry and research institutions and industry and the universities.

Another suggestion to be accepted at the seminar was that research and development centres should be gradually created within industrial enterprises and that the R&D function should be part of feasibility studies and the design of new large plants. To promote applied research in the universities, the Universities Grants Commission and the Pakistan Science Foundation are likely to give liberal financial support for collaborative research, while the Government may help by providing industry with incentives, such as tax exemption, for sponsoring research projects. Research in the universities suffers from inadequate funds; if more money were available, they could work for technological progress as well as pure knowledge.

Dr Salimuzzaman Siddiqui, a chemist, stressed the point that pure and applied research cannot easily be demarcated. So far as problem solving is concerned, priorities cannot be allocated under rigidly defined categories and there should be a massive but carefully phased multidisciplinary programme. Most of the participants felt strongly about another point raised by Dr Siddiqui, namely that Pakistan was spending barely 0.2% of its gross national product on R&D compared to 2-4% in many advanced countries: there was need for a major change. □

CHINA

Desert song

LIKE the United Nations, which organised an intergovernmental conference on desertification in Nairobi earlier this month, China has been taking an interest in the problems of desert control. Over recent years two national conferences on desert control have been held and several major works on aspects of desertification have been published, including 'An outline of Chinese deserts' and 'Summary of mass experience in China's desert control'. Two further works, 'Control of sand destructive to farmland' and 'Protecting railways against sand' are to be published soon.

Using the findings reported in these publications as guidelines, prefectures, counties and communes in six provinces and autonomous regions have drawn up their own plans to combat or even reverse desertification taking into account local conditions. The now established causes of desertification—unplanned irrigation, excessive tree felling, overcultivation, overgrazing, with drought being a condition but not a fundamental cause—are supported in ancient Chinese records. Measures taken to tame deserts include:

- planting wind resistant shrubs on the windward side of land threatened by encroaching deserts and tall trees near farmland. When the wind blows at average speeds, the length of the protected area is some twenty times the depth of the forest shelter belts. Criss-crossing tree belts in oases provides further protection against the wind.
- creating new oases by building reservoirs to store seasonal flood water from streams and lakes and using melted snow from the mountains or underground water to irrigate the reclaimed land before planting trees to protect it.
- developing pasture land for animal husbandry in deserts with fixed sand dunes or only interspersed shifting sand. Forage bases can be established on flat land in between sand dunes where water resources are relatively abundant.

Such measures are important especially as 11% of China's total land area, about one million square kilometres, is desert. Just over half of this is dune desert, the rest being gravel and other types. The Academia Sinica runs a desert research institute which studies desert distribution and type, the processes which form them, their natural conditions and ways of combating destructive sandstorms and using water and soil resources in sandy areas and in reclaimed land. It also publishes a quarterly journal *Desert Research*. In addition, the state has set up experimental forest farms and twenty demonstration stations for desert control. Many species of plants, which will survive in the desert, have been grown at the stations.

T. B. Tang

IN BRIEF

EEC report

ENERGY, uranium prospecting and the use of weedkillers containing dioxin were topics for questions last week at the European Parliament in Luxembourg. EEC Commissioner, Richard Burke, told the Parliament that the Commission is considering the possibility of including wind energy in the next research programme. The EEC already has plans for building a prototype plant to generate electricity from solar energy and there were suggestions for a comparable unit based on wind energy. However, it was unlikely, according to Burke, that wind energy could be more than peripheral because of the few areas within the EEC where

it would be suitable. Initial results for uranium prospecting in the EEC were reported as encouraging and those for Greenland as good.

There was concern over the use of weedkiller 2,4,5-T, which may contain traces of dioxin, the chemical released around the North Italian town of Seveso following an explosion at a chemical factory. The weedkiller is presently allowed in all EEC countries except Italy, Antonio Giolitti told the Parliament. Limiting its use should be considered following each item of information, he said. The Commission's proposed directive on harmonising safety regulations on dangerous manufacturing industry is to be discussed—

for the third time—by EEC government experts on 13 and 14 October.

Leakey in Europe

To mark the inauguration of its European chapter, the L. S. B. Leakey Foundation has endowed two post-graduate students at St John's College, Cambridge. Named after Dr Louis S. B. Leakey and based in the United States since 1968, the foundation has given more than \$1,000,000 to support the study of man's origins, behaviour and survival. The first task of the new chapter, Dr Bernard Campbell its vice-chairman said last week, will be to raise its own funds mainly to support anthropological research.

A FEW months ago (19 May, page 201) I voiced pessimism about the laetrile movement, but now I am in a more contemplative mood. Eleven State Legislatures have passed measures authorising the sale of laetrile. A most interesting day of reckoning is ahead, because no one, least of all the lawmakers, seems to know what laetrile is. Vials containing the solution are sometimes microbially contaminated. The 'potency' of various samples ranges from 14–87% of claims, and one batch of 'laetrile' capsules was found to consist of dehydrated iced tea, which was probably an improvement, because iced tea, unlike amygdalin ('street laetrile'), does not liberate cyanide in the digestive tract. Dr Gerald Lewis fed experimental animals with laetrile and sweet almonds; the almonds contain beta-glycosidase, which hydrolyzes amygdalin, and the animals died of cyanide poisoning. A child died similarly when she ate her father's laetrile tablets. I pointed this out to a pro-laetrile California state senator, and he told me that aspirin kills, too.

I am somewhat encouraged by the fact that the 'establishment' is finally bestirring itself. The Director of the National Cancer Institute, who opined that Ernst Krebs' theory of laetrile action was a great idea, and he wished it would work, has now departed; the US Surgeon General, Julius Richmond, now says that laetrile is useless and may be dangerous.

The best recent news stories were those concocted by the laetrile proponents at hearings before Senator Edward Kennedy on 12 July. Dr John Richardson, whose licence to practise medicine has been revoked, said that he, Ernst Krebs Jr and Robert Bradford were the victims of a conspiracy

by "the major oil and drug companies, the Food and Drug Administration (FDA), the American Medical Association, the National Cancer Institute, the American Cancer Institute"; he also suggested that the Rockefeller family was involved. The witnesses, including Bradford, were

Iced tea or laetrile?



THOMAS H. JUKES

unable to write the formula for laetrile "on a piece of paper", but Bradford, an engineering technician, said that "orthodox medicine was not qualified" to evaluate clinical tests of laetrile. Krebs, who is appealing a six-month jail sentence for violating his probation by selling laetrile, was reported as seeking to leave the impression that he was promoting laetrile for "purely humanitarian reasons". Carol Hehmyer, who prosecuted him, testified that he and his associates in San Francisco took in more than \$500,000 in two years by "selling laetrile and other outlawed medicines".

The 'victimisation' of Richardson, according to newspaper articles, seems to be rendered tolerable by his income (he grossed \$783,000 in 1974) and his opulent lifestyle. Bantam Books have printed 200,000 copies of his new book, *Laetrile Case Histories*. The FDA caustically pointed out in 'The Federal Register', 5 August, that Richardson's book states that he threw out the cases with "the weakest medical histories" and described only 62 out of 4,000 patients! In his book he also tells how he got the idea of laetrile promotion from Ernst Krebs Jr, whose biochemical theories, exposed as fantasies by Professor David Greenberg in 1974, served to convince Andrew McNaughton Jr, a former Canadian test pilot who is the leading entrepreneur of laetrile in Tijuana.

The scientific exposition by Krebs took place, according to McNaughton, in conversation at a chance meeting in a drugstore in Miami some years ago. Such erudite technicalities were not needed to persuade the legislators of 11 states. They responded to chanting crowds who wore T-shirts emblazoned with the words "Laetrile Works, You Bet Your Life". However, a slim majority of the California State Assembly Health and Welfare Committee was unconvinced. They killed the laetrile-legalising bill by a vote of six to five. The bill had passed the corresponding State Senate Committee unanimously. The author of the bill, Mr William Campbell, who for some reason is a member of the senate committee, says he will try again in January. Perhaps a more useful form of legislation would be a constitutional amendment to inhibit state governments from legalising quack remedies.

correspondence

Detecting dead bodies in 1718 and 1976

SIR,—I reported (*Nature* 262, 816 (1976)) that dead bodies of a dog and a cat were detected by the presence of fruit-bodies of an agaric species (*Hebeloma vinosophyllum*) appearing on the ground above the buried bones, and suggested the possibility that a victim of homicide buried in soil could be located by this or similar fungi. In response to a 'Science report' in *The Times* (2 September 1976) summarising my article, Dr Denys W. Tucker, formerly of British Museum (Natural History), informed me of a passage published in 1718, (Aubrey, J. *The Natural History and Antiquities of the County of Surrey* 111, (E. Curll, London 1918)), which contains the following lines (pages 225–26) written by John Aubrey of his visit to Woking in Surrey.

"The Grave-Digger here told me, that he had a Rule from his Father, to know when not to dig a Grave upon a Corps not rotted; which was, when he found a certain Plant about the Bigness of the Middle of a Tobacco-Pipe, which came near the Surface of the Earth, but never appear'd above it. It is very tough, and about a Yard long; the Rind of it is almost black, and tender, so that, when you pluck it, it slips off, and underneath is red; it hath a small Button at Top, not much unlike the Top of an *Asparagus*: Of these sometimes he finds two or three in a Grave. He is sure it is not a *Fern Root*.

He hath with Diligence trac'd to its Root, and finds it to spring from the Putrefaction of the dead Body. . ."

The phenomenon described here is close to what I suggested, but is not exactly the same as that expected of ammonia fungi including the above-mentioned species (Sagara, N. *Contr. biol. Lab. Kyoto Univ.* 24, 205–276 (1975)). "It" reminds us of the rhizomorph, pseudorhiza, or aborted fruit-body produced by certain fungi, but does not fit those of any known ammonia fungi. Further, if we interpret "the Putrefaction of the dead Body" as putrefying corpse, the ammonia fungi would not appear at such an early stage of decomposition.

I have been discussing the matter with some noted mycologists and botanists from the UK and Japan, but so far we have not succeeded in pointing out any possible organism for the structure described by J. Aubrey. I would appreciate receiving suggestions as to what it might be and any similar information from any part of the world.

I thank Dr Tucker for the information, and Drs Roy Watling, Geoffrey C. Ainsworth, Rokuya Imazeki, Siro Kitamura, Osamu Sinoda, and Minoru Hamada for the discussion.

Yours faithfully,

NAOHIKO SAGARA

Biological Laboratory,
Yoshida College,
Kyoto University, Japan

The twin paradox

SIR,—Tom Wilkie's remark (28 July, page 295) that the resolution of the twin paradox lies in the fact that the equivalence of the twins is destroyed by the necessity for the 'moving' one to reverse his motion continues to ignore the fact, pointed out in my letter in *Nature* of 31 August, 1973, that, if that were so, the equivalence would be maintained during the first half of the journey, before reversal occurs. Hence, in the example there cited, Paul, the 'moving' twin, would reach the distant planet a teenage boy, and the reversal and return would restore him to babyhood. It is not customary in scientific research to ignore such obviously remarkable and important points. Will Mr Wilkie therefore kindly say whether he accepts this necessary consequence of his suggested resolution of the paradox, or agrees with me that it makes such a resolution impossible?

Yours faithfully,

HERBERT DINGLE

Hull, UK

Tom Wilkie replies: I stand by every word of my article and do not accept that the above is a necessary consequence. I am writing to Professor Dingle privately on this matter.



Competition 14 asked for a boring or meaningless paragraph. £10 to E. R. A. Beck of Vienna for these introductory remarks:

The subject to be considered in this session of the symposium is one on which much research has been done, much grey matter has been activated and much fruitful discussion has already taken place. Its importance is well known to all of us here today, whether we face it at the laboratory bench or at an adminis-

trative, policy-making or governmental level. Indeed, the fact that an international organisation has chosen to devote an afternoon to an exchange of information on this topic is sufficient evidence that interest has transcended national boundaries. In some form or other, it affects the developed and the developing countries, the technologically advanced and the less-advanced peoples. There is, as yet, no consensus on a solution, nor indeed on a programme for the coming period. In my talk, therefore, I shall attempt to focus attention on some of the more significant points, and express some humble thoughts of my own as to how we might, together, approach the task of seeking a methodology for tackling this absorbing question, ensuring as we do so that our endeavours will also serve those facing this problem in the nations less well endowed with trained manpower and sophisticated equipment. (Aside, speaker to chairman: "I say, which symposium

is this; 'Advanced techniques in control of the screw-worm fly', or 'Special constraints in seeding methods for MHD generators?')

Honorable mention to J. R. Richards (Tadcaster, North Yorkshire) for a genuine paragraph from *The Mind of Light* by Sri Aurobindo.

Competition 15. The front cover of *Nature* used to carry a quotation from Wordsworth:

'To the solid ground
Of nature trusts the Mind that builds
for aye'.

£10 for the best quotation or verse, serious or humorous, suitable for these days. Entries to Competition 15, *Nature*, 4 Little Essex Street, London WC2 by October 20.

news and views

Intercalation complexes of DNA

from Donald Voet

It has now been sixteen years since Lerman proposed that aminoacridine dyes bind to DNA by intercalating between its stacked bases. Since then, numerous other planar aromatic molecules, many of which are potent drugs or mutagens, have been found to bind to DNA in a similar manner. Yet the detailed geometries of these intercalation complexes have hitherto been only poorly defined because of the low quality of the available fibres of nucleic acids complexed with intercalation agents. The situation has been greatly remedied recently by the elucidation of several X-ray crystal structures of self-complementary dinucleoside phosphates in complex with various intercalating agents.

X-ray studies have shown that self-complementary dinucleoside phosphates such as cytidyl-(3',5')-guanosine [CpG] often crystallise as miniature double helices. By this it is meant that the dinucleoside phosphate forms two symmetry-related Watson-Crick base pairs with a neighbouring molecule and that their sugar-phosphate backbones have the conformational parameters found in a double helix. In an intriguing extension of this work Sobell and his coworkers have recently reported the X-ray structures of several intercalation complexes of self-complementary dinucleoside phosphates. These are the 2:2 complex of ethidium with 5-iodouridylyl-(3',5')-adenosine [iodoUpA] (Tsai *et al.* *J. molec. Biol.* **114**, 301; 1977); the 2:2 complex of ethidium with 5-iodocytidylyl-(3',5')-guanosine [iodoCpG] (Jain *et al.* *J. molec. Biol.* **114**, 317; 1977); the 2:2 complex of 9-aminoacridine with iodoCpG (Sakore *et al.* *Proc. natn. Acad. Sci. U.S.A.* **74**, 188; 1977) and the complex of proflavine with iodoCpG (Seshadri *et al.* *Abstracts Amer. Crystallogr. Assoc. Meeting, Michigan State Univ.* 1977). These complexes, which are all heavily hydrated, have quite different crystal structures from one another. Yet their molecular structures show striking

similarities. In all these structures the dinucleoside phosphates form miniature double helices that intercalate an ethidium or an acridine molecule. Thus the base pairs in these structures are separated by about 6.8 Å which is twice the usual base stacking distance found in the B form of DNA (the usual native form).

The intercalation agents are accommodated in the complex by the unwinding of the miniature double helices from the conformations found in nucleic acid double helices. In doing so all of these dinucleoside phosphates have adopted a rather distinctive sugar puckering pattern in which the ribose sugar of the pyrimidine nucleoside is in the C3' *endo* conformation and that of the purine nucleoside is in the C2' *endo* conformation. The same C3' *endo*-(3',5')-C2' *endo* puckering alternation has been observed in the pseudo intercalation complex of 9-aminoacridine with ApU (Seeman *et al.* *Nature* **253**, 324; 1975). (In this structure the bases pair in a Hoogsteen arrangement without double helix formation. However the base pairs alternate with 9-aminoacridine molecules as in the foregoing intercalation complexes.) Furthermore, computerised model building studies by Alden and Arnott (*Nucleic Acids Res.* **2**, 1701; 1975) have suggested that the most satisfactory conformation for an intercalation complex of B-DNA incorporates the above puckering alternation.

It has long been known that, at saturating concentrations of intercalating agents, DNA will intercalate one molecule for every two base pairs. This observation has led to the 'neighbour exclusion' model which states that the binding of intercalation agents to DNA is subject to the restriction that adjacent binding sites be unoccupied. This model is neatly rationalised by the requirement of a C3' *endo*-(3',5')-C2' *endo* pucker alternation at the intercalation site. Such an alternation can maximally occur between every second base pair.

The foregoing crystal structures account for solution studies which indicate that ethidium preferentially

binds to sequences of the type pyrimidine-(3',5')-purine. In the above structures there are extensive stacking interactions between the nucleic acid bases and the intercalating molecules. However, model building studies show that in complexes which have these base sequences reversed the degree of ring overlap is much reduced. Presumably these latter complexes are less stable than the former. This result also explains why no intercalation complexes of ApU or GpC have been crystallised.

The various structural features common to the foregoing intercalation complexes, which include an 8° relative tilt of the base pairs towards the wide groove, have prompted Sobell *et al.* (*J. molec. Biol.* **114**, 333; 1977) to propose a detailed model for intercalation complexes of B-DNA. In this model, which is substantially based on computer-generated model building, the helix axis is kinked about the intercalation site by 8° and is unwound by 10°. This latter figure is in reasonable agreement with the results of solution studies of intercalation complexes. In addition the sugars about the intercalation site have the pucker alternation described above.

The structural features of this model have led Sobell *et al.* to extend their model building studies to generate a general model for the kinking of B-DNA. In this model the helix axis is bent by 40° into the wide groove through a conformational change that is made possible by a C3' *endo*-(3',5')-C2' *endo* pucker alternation about the kinking site. Then, depending on the interval along the helix axis of these kinks, various families of supercoiled DNA can be generated and Sobell *et al.* present several striking computer generated drawings of these superhelical DNAs. Perhaps the most interesting of these is the one in which a kink occurs every 10 base pairs. This gives rise to a left-handed superhelical structure that contains 1½ turns per 140 base pairs and has an axial length of 80 Å. These and other structural features make it an attractive model for the DNA in nucleosomes.

The foregoing models result from the great similarities among the four crystalline intercalation complexes reported by Sobell and coworkers. But into many ointments a fly will fall. In this case the fly is in the guise of the crystal structure of a 3:2 complex of proflavine with CpG that is reported by Neidle *et al.* in this issue of *Nature* (page 304). This structure, which results from a collaboration between researchers at Kings College, London, and The Institute of Cancer Research, Philadelphia, consists, in part, of miniature double helices into which proflavine molecules are intercalated. The spacing between adjacent C:G base pairs is the expected 6.8 Å. But what is unexpected and potentially damaging to the plausibility of Sobell's models is the fact that all the sugars are in the C3' *endo* conformation rather than having the C3' *endo*-(3',5')-C2' *endo* pucker alternation. Furthermore the miniature double helix is not unwound relative to the helical conformation of uncom-

plexed RNA-11. Rather, the miniature double helix accommodates the intercalated proflavine molecule by a stretching distortion involving small changes in the backbone and glycosidic torsion angles.

It may be that the structure reported by Neidle *et al.* is simply a red herring. After all, a dinucleoside phosphate could hardly be expected to behave as rigidly as a polynucleotide, especially in its interactions with other molecules. But the same criticism can be levelled at the structures reported by Sobell and coworkers. Furthermore, all of the dinucleoside phosphates discussed here are composed of ribonucleotides and therefore cannot be considered to be ideal models for DNA. (The fact that acridine fluoresces red when complexed with RNA and green with DNA indicates that the acridine molecule senses a difference between RNA and DNA). What is clearly necessary to settle the question of the conformation of an intercalation complex of DNA is the

crystal structure of an intercalation complex of a DNA fragment that is at least four base pairs in length. Rumour has it that there are several groups now attempting to crystallise such a complex.

In a wider sense these studies have shown that 'small molecule' crystallography continues to be a valuable tool in elucidating biological structures. The structures discussed here all contain several molecules in their asymmetric unit and hence are very large according to present day standards for 'small molecules'. Indeed, the asymmetric unit of the 9-aminoacridine: iodoCpG complex, which contains 248 non-hydrogen atoms, approaches the size of some of the smaller proteins whose structures were solved using 'macromolecular' crystallographic techniques (rubredoxin for example has 401 non-hydrogen atoms). Thus it seems that the differences between 'small molecule' and 'macromolecular' crystallographic techniques may soon grow indistinct. □

Elementary particles—a rich harvest

from David J. Miller

A symposium on Lepton and Photon Interactions at High Energies was held in Hamburg on 25–31 August, 1977.

A SCIENTIFIC journalist recently tried to persuade me that high-energy physics was one of the fields of science, and there might be others such as molecular biology, where enough progress has been made for the moment. He could not have maintained such an attitude if he had been at this year's conference. The momentum of progress in the field is unabated, with new and exciting experimental data being matched by a significant growth in theoretical understanding.

Evidence for a fifth quark?

The most exciting result of all came from L. Lederman, whose Columbia Fermilab group have now not only confirmed the Υ (upsilon) bump which they see in muon pairs from proton-proton collisions, but have shown that it is composed of at least two clear resonances, one produced three times more strongly than the other. It is almost certainly another pair of objects like the J/ψ and the ψ' ; but this time the theorists were ready for it. There is even a name for this whole class

of particles—'Onium'. Positronium—the pseudo atom made up from an electron and its antiparticle the positron—has been with us for many years. The ψ and ψ' have been called 'charmonium' because they behave as if they are made of a charmed quark and antiquark. A considerable amount of new evidence was reported on the level-structure of charmonium—not only the familiar J/ψ and ψ' 3S states which couple to e^+e^- by way of a photon, but the 3P states which lie between, and the rather less well established 1S states. A surprise has come as the theories have been refined. The non-relativistic theory of heavy quarks in a potential well had sufficient input from the 1^3S (J/ψ), 2^3S (ψ') and 1^3P that it was possible to predict a 3^3S state above the ψ' , at 3,770 MeV/ c^2 —just above the threshold for pair-producing the charmed D mesons. Experimenters at the e^+e^- storage rings SPEAR in California and DORIS in Hamburg had not investigated this region very carefully because of interest in the complex structure just beyond it from 4,000 to 4,450 MeV/ c^2 . They went back to look for the 3,770 only a few months ago and it was found as a clear signal exactly where predicted. If the upsilon is the next onium then it must be made from a pair of even heavier quarks than the charmed quark, so non-relativistic

potential theory should work even better in this case. As soon as Lederman's talk ended the theorists got out their pencils and paper, and in a talk a few hours later K. Gottfried was able to show that all of the known properties of the upsilon could be accommodated in an onium picture. There is therefore almost certainly a new quark flavour in addition to the everyday 'up' and 'down' quarks, the strange quark and the charmed quark.

More on charm

It is only about a year since it became clear that we had indeed seen charmed particles—the D mesons produced in e^+e^- annihilations. Many new properties of the Ds were reported at Hamburg and also evidence for their strange and charmed relative the F. The pseudoscalar Ds had been hard to separate from the vector D* mesons which decay directly into a D and a pion, but the discovery of the 3,770 has brought an unexpected bonus. It decays strongly into $D\bar{D}$ pairs, but it is not massive enough to give D*. One can therefore set the energy of the e^+e^- rings at 3,770 and study the rather complicated weak decays of the Ds without any extraneous pions. The other approaches to producing charm have been making slower progress. Neutrino experiments at Fermilab and at CERN are now accumulating large

numbers of events with two muons of opposite sign in the final state, or with a muon and an electron. All properties of these events seem to be consistent with charm's being produced in about 5% of neutrino interactions. So far no wholly satisfactory result has been reported on the production of charmed baryons. The Columbia-Fermilab experiment by Wonyong Lee's group has a clear signal with antilambda and three pions. They should also see something with lambda and three pions but they do not, so the significance of the antilambda signal must be questioned. It seems likely that the bubble chamber neutrino experiments will be the first to establish the existence of charmed baryons, but the results from several groups on strange particles from charmed particle decay are fluctuating wildly due to small statistics and no clear conclusions can yet be drawn.

More signs of τ

For two years now there have been signs from SPEAR and from DORIS that the increase of e^+e^- cross sections at about 4 GeV/ c^2 is due to two thresholds, not just to the charm threshold. M. Perl, using SPEAR, was the first to identify a class of events which have a muon and an electron with opposite signs and in which a great deal of the energy escapes as unseen neutral particles. New detectors at SPEAR and new data from DORIS have confirmed the existence of these events and it looks increasingly convincing that they are caused by a new heavy lepton, the tau (τ), a big brother to the electron and the muon. In particular, the cross section for producing them seems to rise smoothly and steadily from threshold towards the correct asymptotic value for the pair production of spin- $\frac{1}{2}$ particles, with mass about 1,850 to 1,900 MeV/ c^2 , coupled to the photon in the normal way. There was some excitement early in the conference when it seemed that one of the most easily predicted decays of the τ (to a pion and a neutrino) was not seen, but the experimenters looked more carefully at their data and were eventually convinced that there was no anomaly.

Neutrino physics

In neutrino physics the new results from the CERN SPS have come at the same time as the second generation results from Fermilab, with the consequence that some of the odder effects suggested by single experiments in the past can now be seen to have gone away. In particular the Harvard - Pennsylvania - Wisconsin - Fermilab, 'HPWF', suggestion of a threshold effect at neutrino energy of about 40 GeV has not been confirmed by any

Has the *src* gene product been found?

from Robin Weiss

AVIAN sarcoma viruses carry a gene, *src*, which is required for the transformation of fibroblasts and the induction of sarcomas, but which is superfluous for virus replication. Because mutants of *src* have been isolated that are temperature sensitive for the maintenance of the transformed state of the cell, it is widely assumed that *src* codes for a transforming protein. On page 346 in this issue of *Nature*, Brugge and Erikson report the detection of a transformation-specific antigen which appears to be a good candidate for the *src* protein. Sera obtained from rabbits bearing tumours induced by avian sarcoma virus (ASV) react with a protein of 60,000 daltons present in transformed cells, which is unrelated to viron structural proteins. The same protein is found in ASV-transformed avian and mammalian cells, strongly indicating that it is a virus-coded protein. Cells infected with transformation-defective, *src* deletion mutants of ASV do not express this protein; neither could it be immunoprecipitated from cells infected with temperature-sensitive *src* mutants and incubated at the non-permissive temperature.

This is not the first time a transformation-specific antigen has been identified in ASV-transformed cells. There is an extensive literature (reviewed by Kurth *Biomembranes* 8, 167; 1976) on a tumour-specific cell surface antigen (TSSA) to which Brugge and Erikson make no reference. It will be important to see whether the 60,000 dalton protein is related to TSSA.

Several attempts have been made to

identify the *src* gene product by *in vitro* translation of ASV RNA. Pawson, Martin and Smith (*J. Virol.* 19, 950; 1976) were not able to identify any polypeptide present in translation products of the ASV genome that was absent from those of a transformation-defective (*td*) mutant. Two recent reports, however, each claim that two polypeptides of approximately 18,000 and 25,000 daltons can be detected after translation of ASV RNA but not of *td* ASV RNA in the reticulocyte system (Kamine & Buchanan *Proc. natn. Acad. Sci. U.S.A.* 74, 2011; 1977; Beemon & Hunter *Proc. natn. Acad. Sci. U.S.A.* 74, 3302; 1977). The work of Beemon and Hunter is particularly interesting because they have demonstrated by analysis of tryptic peptides that the two polypeptides absent in *td* ASV translation products are unrelated to the precursor polypeptide of the viron core proteins. Since *src* is located near the 3' end of the ASV genome it is not easily translated from undegraded genomic RNA preparations, but selection of smaller fragments containing the poly(A) at the 3' end of the molecule proved effective for the translation of these presumed *src* products.

The antiserum that reacts with the 60,000 dalton polypeptide should be useful for selective precipitation of *in vitro* translation products. In their report, Brugge and Erikson already quote a further paper in press on that subject.

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other group. But there are new effects on a more subtle level which are beginning to be believed because they are seen in so many experiments using very different techniques. Until now it has been usual to interpret neutrino data in terms of the simplest quark-parton picture. This will clearly not work any longer. For some years it had been clear that electron-proton and muon-proton scattering data required important corrections to the simple quark model. Now the same corrections have been shown to apply to the charged current neutrino data. Theorists are very pleased about this, because the kind of correction required is just what would be expected from the more formal theory of quarks—quantum chromodynamics 'QCD'.

Neutral current neutrino data has also been improving rapidly, and it is clear that neutral current interactions producing strongly interacting particles (hadrons) are very well described by the Salam-Weinberg GIM model (see *News and Views* 264, 398; 1976) otherwise known as $SU(2) \times U(1)$. P. Sandars (University of Oxford) gave a status report on the other end of the neutral current field—the search for the atomic effects of interference between the weak neutral current and the electromagnetic interaction. Here there are problems. Sandars's group and a group at Washington State University have performed optical rotation experiments with laser light passing through bismuth vapour. If the Weinberg-Salam model is true, and

has the parameters determined from the hadronic neutral-current experiments, then they should have seen large effects due to these parity-violating interferences. But neither group sees any effect, and they have spent a great deal of time checking both their experimental sensitivity and the theory in order to be sure (*News and Views* 264, 505; 1976).

S. Weinberg and others discussed the meaning of these results. It seems that $SU(2) \times U(1)$ is to the weak interaction what the naive quark-parton model has been to QCD; a first approximation which has fitted a surprisingly large part of the data. Now it will be necessary to enlarge the model to accommodate the new quarks and leptons, the absence of atomic neutral currents and perhaps also whatever it is that is causing trimuon events. The HPWF group still sees these events, though the big new CERN neutrino experiment is not yet claiming a definite effect. There is particular interest in two of the HPWF events in which all three of the muons have been given very large energies. This suggests that they have all come from the 'neutrino end' of a neutrino-nucleus interaction—something which no one was able to explain satisfactorily in the framework of current weak-interaction theory.

Plans for the future

The last morning was devoted to the future ('and its alternatives', a phrase quoted by W. Panofsky of SLAC from a US Government memorandum). Panofsky, and H. Schopper of the host laboratory, reviewed the plans for their laboratories and for their e^+e^- storage rings PEP and PETRA which will start work at about 30 GeV in the centre of mass (15+15 GeV beams) in 1979 and 1978 respectively. There was a fascinating talk from J. W. de Wirc of Cornell University on CESR, the 8+8 GeV e^+e^- storage ring, which is being built on a minute budget by the standards of the big laboratories. Cornell is one of the last universities in the world to be able to build its own high energy physics facilities. The record of achievement on their 10 GeV electron synchrotron has been excellent and they are now especially confident of doing a good job with CESR because the upstart falls right in the middle of their useful range of energies. Schopper described how the energy of DORIS will be extended to reach the upstart, but the commitment to build PETRA on a very rapid schedule makes it impossible to do this immediately. The usefulness of having more than one laboratory doing e^+e^- physics at the same energy has been illustrated repeatedly not only by the results from SPEAR and DORIS on charm and τ production but also by

the latest results from the lower energy French and Italian machines, DCI and ADONE, which are still a long way from having cleared up the problems which remain in the energy region below 3.1 GeV. This region was left almost untouched in the rush to discover the 'new physics' and the Onium-structure of the old quarks—up, down and strange—is still not understood. These two machines will have to collect much more data before we can hope to sort it out.

Although our ideas of QCD and the weak interaction still leave many questions unanswered, J. D. Bjorken of Stanford drew attention in the final talk to the major discoveries which can be confidently predicted for the next 10 or 20 years. As energies rise, the old fashioned strong interaction and the electromagnetic interaction will have a decreasingly important role compared with the quark-gluon coupling of QCD and the charged and neutral currents of weak interactions. As well as discussing the zoo of new objects which might be produced as this regime is reached, he compared the merits of different types of machine which have been proposed to get there. The clear leader is the large e^+e^- machine LEP, as requested by the European Committee on Future Accelerators (*see News and Views* 267, 12; 1977). It would provide data on almost every major question in particle physics, and would also be able to sit at the mass of the neutral current intermediate boson and become a Z^0 factory—mass producing the Z^0 boson in a resonant mode so that the Z^0 couplings to all the quarks and leptons can be investigated simultaneously. Next in usefulness come high energy pp storage rings. These will give us an idea of effects beyond the range of extrapolation of our theories and at energies which other kinds of machine cannot reach. Cosmic rays have given hints of odd behaviour at tens of thousands of GeV (tens of TeV), but it will be storage rings such as ISABELLE at Brookhaven New York, rumoured to be about to be funded in the next ERDA budget, which will make it possible to take such effects seriously. Fixed target proton machines such as the SPS and the Fermilab machine will always be needed to provide neutrino, muon and hadron beams. The Russians are planning a 3 TeV machine and Fermilab's 1 TeV machine is still going ahead—now named the 'Tevatron'.

In the light of Bjorken's discussion there was some disappointment among many physicists at the current plans for the CERN laboratories near Geneva. The only new project which is being asked for by the directorate is an anti-proton on proton colliding beam facility to be added to the SPS. Yet it

is now well known that the antiquark content of the proton is sufficiently large that a high flux pp machine like ISABELLE should produce much more quark-antiquark physics than any conceivable pp machine. The other suggested development for the SPS, an electron ring called CHEEP (though it will cost a little more than the anti-proton facility), has a great deal more theoretical backing. The quark parton model was evolved in response to the first deep-inelastic electron proton scattering results. If CERN builds CHEEP it will have a unique tool to investigate many anticipated high energy effects by way of deep inelastic processes which are much easier to understand than quark-antiquark scattering. If CERN does not build CHEEP then it is possible that a proton ring could be added to PETRA instead, but it seems improbable that Germany would maintain its present level of subscription to CERN if it had such an advanced home-based programme.

Despite the inevitable arguments about tactics and despite the knowledge that funds will be hard to find if economic crises continue, there is confidence that the machines after PEP and PETRA will be built within a reasonable period. Bjorken mentioned something which particle physicists feel very strongly; the case for building the next generation of accelerators can be made more precisely and with more confidence now than has been possible for any previous generation of accelerators. Here is a field where progress must continue. □

Recognition of lysosomal enzymes

R. Colin Hughes

IN the late 1960s, Elizabeth Neufeld and her colleagues showed that 'corrective factors' secreted by skin fibroblasts established from normal individuals could eliminate intracellular deposits of mucopolysaccharides that build up in mutant cells established from the skin of mucopolysaccharidoses patients. Such patients lack the particular lysosomal enzymes to catabolise these substances in the normal way (see Neufeld *et al.* *A. Rev. Biochem.* 44, 357; 1975; Dawson & Lenn in *Handbook of Clinical Neurology* 27, 143; 1976). Later the 'corrective factors' were identified as precisely those lysosomal enzymes missing in each of the mutant cell lines (Bach

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et al. Proc. natn. Acad. Sci. U.S.A. **69**, 2048; 1972; Cantz *et al. J. biol. Chem.* **247**, 5456; 1972; von Figura & Kresse *Biochem. biophys. Res. Commun.* **48**, 262; 1972). More recently, Sly *et al. (J. Pediat.* **82**, 249; 1973) reported a child with a mucopolysaccharide storage disease due to a deficiency of β -glucuronidase whose fibroblasts were corrected by purified bovine or human enzyme (Hall *et al. Archs Biochem. Biophys.* **155**, 32; 1973; Brot *et al. Biochem. biophys. Res. Commun.* **57**, 1; 1974). Since lysosomal enzyme deficiencies produce severe problems for the patients the encouraging idea of an enzyme replacement therapy prompted by these experiments has been pursued vigorously. Such efforts are likely to be aided by the impressive progress being made in showing how lysosomal enzymes added externally to cells find their way to the abnormal intracellular storage materials.

This process involves specific pinocytosis and apparently utilises part of the normal life cycle of these enzymes, which according to Hickman and Neufeld (*Biochem. biophys. Res. Commun.* **49**, 992; 1972) are first secreted to appear on the outside of cells and are then quickly taken up again by the same or adjacent cells to perform their enzymic function. Just why the lysosomal enzymes go through this complicated scheme is completely unknown. The important point is that one step in the proposed scheme involves recognition by fibroblast cell surface receptors. Some of the molecular features of this recognition are becoming clearer and there are several surprises.

The removal of carbohydrate from human β -hexosaminidase, which like most lysosomal hydrolases is a glycoprotein, was found to abolish its uptake by fibroblasts and led to the idea that recognition of the enzyme by fibroblasts involves a carbohydrate moiety (Hickman *et al. Biochem. biophys. Res. Commun.* **57**, 55; 1974). The classic work of Ashwell and Morell had already shown that carbohydrate specificity was critically involved in recognition of glycoproteins by mammalian liver, a reaction that explained the rapid clearance of these glycoproteins from the circulation. The properties of the liver cell receptor are such that only glycoproteins with terminal galactose residues are recognised and taken up by the hepatocyte (see Ashwell & Morell *Trends in Biochemical Sciences* **2**, 76; 1977 for recent discussion). Although the concept is clearly similar this receptor is sharply distinguishable from the receptor on fibroblasts which mediates the uptake of lysosomal hydrolases. The clearest evidence on the molecular

detail of this interaction so far has been obtained by Sly and his colleagues using human β -glucuronidase.

Previous studies (Glaser *et al. Archs Biochem. Biophys.* **166**, 536; 1975; Brot *et al. Biochem. biophys. Res. Commun.* **57**, 1; 1974) had shown that the enzyme appeared in two forms separable by a charge difference. Only the more acidic form, concentrated particularly in platelets but relatively scarce in placenta, was taken up by skin fibroblasts established from the patient with β -glucuronidase deficiency. At the recent ICN-UCLA meeting on Cell Surface Carbohydrates and Biological Recognition in Keystone, Colorado, Sly reported (*J. supramol. Structure Supplement* (1977) in the press) the competitive inhibition of β -glucuronidase uptake by certain yeast mannans containing phosphate and by simple hexose phosphates, in particular D-mannose-6-phosphate. Furthermore, the high uptake form of the enzyme was converted to a less acidic low uptake form without loss of enzymic activity by treatment with highly purified alkaline phosphatase. More detailed reports (Kaplan, Archard & Sly *Proc. natn. Acad. Sci. U.S.A.* **74**, 2026; 1977; Kaplan, Archard & Sly *J. clin. Invest.* (1977), in the press) substantiate these suggestions for a novel receptor on fibroblasts that recognises hexose phosphate on the lysosomal glycoproteins β -glucuronidase, β -hexosaminidase and β -galactosidase. The same kind of recognition is involved in the uptake of acidic forms of β -glucuronidase from liver, urine, spleen and placenta and the hexosaminidase secreted by normal human fibroblasts also seems to contain the phosphohexosyl group for the fibroblast receptor.

Phosphorylated sugars are very rarely found in mammalian glycoproteins. The sensitivity to alkaline phosphatase suggests a phosphomonoester, similar perhaps to some forms of haemoglobin which contain terminal D-glucose-6-phosphate residues (Haney & Bunn *Proc. natn. Acad. Sci. U.S.A.* **73**, 3534; 1976). Davis *et al. (FEBS Lett.* **65**, 3035; 1976) recently reported the isolation of phosphoglycoproteins from rat brain and demonstrated phosphomannosyl residues. Although phosphomannosyl is a well known constituent of yeast glycoproteins, this is the first example found in mammalian glycoproteins.

Other workers (Hieber *et al. Biochem. biophys. Res. Commun.* **73**, 710; 1976; Fedn *Proc.* **36**, 653; 1977) have been interested mainly in β -galactosidase and believe mannose also regulates uptake of this enzyme by fibroblasts. Whether the mannose residues are phosphorylated is not yet clear. Hieber *et al.*, concluded that this

was a distinct uptake system different from that which mediates β -glucuronidase uptake. Hieber *et al.* have, however, shown that the glycopeptides (carbohydrate chains containing just one or two amino acid residues) inhibit assimilation of β -galactosidase by fibroblasts. The exciting implications of these findings are that such glycopeptides could serve as recognition markers to get proteins rather specifically and efficiently into cells and perhaps opens up the possibility of enzyme replacement therapy in diseases in which a 'high uptake' human enzyme is not readily available.

Several questions concerning the role of the fibroblast surface receptor in lysosomal organisation are raised by these findings. It has been known for many years that β -glucuronidase for example is bound to the inner surface of the lysosomal membrane. Is the same recognition marker used for this interaction as that involved in pinocytosis? This is a distinct possibility since the inner surface of the lysosomal membrane (presumably carrying the specific receptor protein) appears on the outer surface of the cell during lysosomal discharge in the model of Hickman and Neufeld (*op. cit.*). Second, does the fibroblast surface receptor play a part in controlling the levels of extracellular and potentially lethal lysosomal enzymes? This may be so but collateral evidence has revealed yet another recognition system which, like that already discussed, involves mannose-rich carbohydrate chains of glycoproteins but does not require these residues to be phosphorylated.

When rat lysosomal enzymes are injected intravenously in rats they are cleared from the circulation within a few minutes (Stahl *et al. Nature* **264**, 86; 1977; *Proc. natn. Acad. Sci. U.S.A.* **73**, 4045; 1976) and end up in the liver. The system is clearly different from the clearance of galactose terminal glycoproteins, desialysed orosomucoid, by the Ashwell-Morell system which resides in hepatocytes, since there is no inhibition of lysosomal enzyme clearance by orosomucoid unless both terminal sialic acid and galactose residues are removed to expose N-acetylglucosamine terminals. According to Sly *et al. (Fedn Proc.* **36**, 653; 1977; *Biochem. biophys. Res. Commun.* **77**, 409; 1977) clearance of human placenta β -glucuronidase in the rat was similar to that reported by Stahl *et al. (op. cit.)* for rat enzymes, and the liver clearance system was shown to involve Kupffer cells rather than hepatocytes. This clearance system was inhibited even better by mannose terminal glycoproteins than by N-acetylglucosamine terminal glycoproteins. Clearance of N-acetylglucosamine terminal glycopro-

teins was antagonised by simultaneous injection of mannose terminal glycoproteins and *vice versa*, suggesting that there are unlikely to be independent Kupffer cell receptors responsible for clearance of mannose or N-acetylglucosamine-terminal glycoproteins respectively. Presumably the Kupffer cell receptor has relaxed specificity and binds to mannose residues present in both glycoprotein types. It has recently been suggested that the hepatocyte receptor also does not discriminate between galactose and glucose terminals in some circumstances (Stowell *et al.* *Fedn Proc.* **36**, 653; 1977). In this property the mammalian receptors resemble the plant lectins which in general also bind to several different sugars. Since both forms of β -glucuronidase are efficiently cleared from the circulation, the Kupffer recognition system does not require a phosphorylated site on the lysosomal enzyme, raising fascinating speculation on the potential role of phosphorylation in

deciding the circulatory fate and target cell of glycoproteins.

In summary, therefore, the original findings of Ashwell and Morell have opened up to reveal a remarkable collection of cellular receptors for glycoproteins. These receptors differ in specificity according to cell type, for example the preference of the mammalian hepatocyte receptor for galactosyl terminals, the Kupffer and fibroblast cell receptors for mannosyl residues and phosphomannosyl residues respectively while the identification of the avian hepatic receptor as a binding activity for N-acetylglucosaminyl terminated glycoproteins (Lunney & Ashwell *Proc. natn. Acad. Sci. U.S.A.* **73**, 341; 1976) warns against extrapolation across species. The list almost certainly will continue to grow and underlines the feeling that carbohydrate structure is involved in a variety of recognition systems, and defects in such recognition may correlate with some human disease states. $\square$

Diffusion in polymers

from Paul Calvert

SEMICRYSTALLINE polymers behave as if they consisted of two phases, crystal and amorphous, arranged in alternate layers. Most simple properties are a function of the fractional crystallinity but are insensitive to the structure or arrangement of the phases. Some recent measurements on the diffusion of chain molecules in polyethylene serve as a reminder that diffusion can tell us about structural details.

According to the two-phase model a semicrystalline polymer consists of plate-like crystals of about 10 nm thickness separated by amorphous regions of the same size. The crystals have the same properties as hypothetical large crystals and the amorphous material should be the same as completely amorphous polymer. This idea works well for bulk properties such as density, modulus, heat capacity and expansion coefficient (see Wunderlich *Macromolecular Physics I*, Academic Press, 1973). There are probably crystal defects and special crystal surface structure but they do not affect bulk properties.

It seems reasonable that motion in the amorphous phase will be limited by the crystals, but in polymers such as polystyrene and polyethylene terephthalate I am not aware of any evidence for a change in glass transition temperature or mobility between com-

pletely amorphous and partly crystalline polymers. Boyer has suggested that this happens in polyethylene (*Macromolecules* **6**, 288; 1976) but the situation is still confused. Intercrystalline links are chains running from one crystal to another stitching the structure together (Keith & Padden *J. appl. Phys.* **42**, 4585; 1971). These should be more frequent in quickly cooled samples where the crystals are small and have proved useful in explaining effects of molecular weight and cooling rate on mechanical properties. Unfortunately, no very convincing way of detecting them in normal samples has been found (although see Wunderlich *Makromol. Chem.* **175**, 977; 1974). So, although strength and deformation behaviour do depend on structural factors other than crystallinity, and although likely factors have been discussed, there is not much supporting evidence. Diffusion measurements could be very informative about this.

Many measurements of diffusion of gases in polymers were made in the 1950s and early 1960s when plastic packaging was becoming important (see Crank & Park *Diffusion in Polymers* Academic Press, 1968; Rogers in *Physics and Chemistry of the Organic Solid State* **2**, Interscience 1965). It is probably these workers whom we should thank for the disappearance of the soggy potato crisp. In semicrystalline polymers it was found that gas solubilities were proportional to the

amorphous fraction, implying that gases are excluded from the crystals. Michaels and coworkers (*J. Polymer Sci.*, **41**, 53; 1959; **50**, 393, 413; 1961) described diffusion by $D = D_a/\tau\beta$ where D_a is the diffusion coefficient in pure amorphous material, τ is a 'tortuosity factor' dependent on crystallinity and β is an immobilisation factor which is dependent on the size of the diffusing molecule. They initially found that in branched and Ziegler polyethylenes τ was proportional to amorphous content to a power between -1 and -2 rather than the expected inverse proportionality. In later measurements on linear polyethylene (*J. appl. Phys.* **35**, 3165; 1964) they did find diffusion to be simply proportional to amorphous content in samples crystallised by cooling. By annealing, however, they could produce samples of the same crystallinity but with different diffusion rates. This topic has essentially remained in this state since.

Klein and Briscoe made measurements of the diffusion of long chain amides in polyethylene (*Nature* **257**, 386; 1975; *Polymer* **17**, 481; 1976), and have recently extended this to study morphological effects on the diffusion of chain molecules (*Nature* **266**, 43; 1977; *J. Polymer Sci. (Phys.)* (in the press)). They envisage the diffusion of a chain molecule in the interlamellar regions as the motion of a large snake across the floor of a forest, winding amongst the intercrystalline links. This is based on the ideas of de Gennes (*J. Chem. Phys.* **55**, 572; 1971; *J. de Physique* **36**, 1199; 1975), and monte carlo calculations for two dimensional snake-like diffusion ('reptation') around obstacles by Doi (*J. Phys. A* **8**, 417; 1975). With some rather sweeping assumptions about the frequency of intercrystalline links they are able to calculate relative diffusion rates for two compounds containing 45 and 25 carbon atoms in rapidly and slowly cooled samples. The calculated ratios are in respectable agreement with experiment. The calculated and observed diffusion rates are faster in the more crystalline, slowly cooled, sample in contradiction to the results of Michaels for gases, and to simple crystallinity theories.

At the moment this is a lot of theory based on little data, and there are many unanswered questions including why annealed samples are different from continuously cooled ones, why Eby found more rapid diffusion in film surfaces (*J. appl. Phys.* **35**, 2720; 1964) and whether diffusion is homogeneous throughout the spherulites. Nonetheless, a quantitative theory is much better than a qualitative model and we should learn a great deal about morphology in proving it right or wrong. $\square$

review article

Mathematical models in applied ecology

Gordon R. Conway*

Since the 1950s there has been a growing interest in mathematical models as guides to harvesting fish and game or controlling pests and vector borne disease. So far there are few indications that we can use such models in day-to-day ecological management, but experience confirms that they have a powerful role to play in developing management strategies and in the formulation of policy.

CONTEMPORARY research in applied ecology can be criticised on several counts: it is predominantly empirical, the effort is fragmented into different fields and there is little attempt to link economic and social factors with ecological factors. These criticisms are by no means new. That they have to be repeated is a reflection of the considerable complexity of most problems in applied ecology and the very real intellectual and practical difficulties of integrating principles derived from pure ecology with principles arising out of the social sciences.

In the 1960s Watt laid the foundations for a coherent theory of applied ecology¹. His starting point was the perception that all natural resource management fields are related to one another by common dependence on the science of ecology, and by a common concern with the problem of optimisation. He also recognised that they are universally characterised by processes such as dispersal, predation, competition and weather effects whose mathematical properties are typically non-linear and complex. The development of a theory thus also required the elaboration of a common set of analytical tools and mathematical models.

Watt's pioneer efforts stimulated the development of a wide range of applied ecological models²⁻⁶. For the most part these, and earlier models, have been aimed at answering questions of strategy. Built on knowledge derived from the study of undisturbed organisms in their natural environment, they have been directed at the strictly ecological problems of maximising harvests of domesticated or wild animals and plants and minimising populations of pests and diseases. Underlying their development there has often been a conscious search for those natural thresholds and non-linearities in the dynamics of populations which can be successfully exploited for harvesting or control. At their best these models have become powerful didactic tools, guiding research and determining policy.

Strategic models, however, are purely ecological in orientation with no economic and social component. True policy models, which explicitly include these components, have so far been developed largely for fisheries, although in recent years they have begun to appear in the field of pest control. They are, of course, considerably more complex than strategic models but the few successful examples graphically demonstrate the critical importance of understanding the consequences of the interaction between the biological non-linearities of a system and those introduced by economic factors.

More recently, a third kind of model has appeared bearing on the day-to-day decisions involved in the harvesting or control of a specific population. Such tactical models have the formidable task of coping in some detail not only with temporal and spatial heterogeneity, but also with the weather variables which tend to be dominant at the tactical level. As a consequence most are

formulated as computer simulations. So far, very few, if any, have shown practical success.

Together, strategic and policy models may be able to correct the deficiencies of much of today's applied ecological research, by providing a stronger theoretical base, a sense of coherence between the different fields and an understanding of the nature of the policy questions and their answers. That may in turn lead to tactical models for use in everyday management.

This review begins with a brief historical note, which is followed by a discussion of the relative merits of small and large scale modelling in applied ecology. The main body of the review examines the relative progress of policy, strategic and tactical models in the harvesting of fish and game and the control of pests and vector borne disease.

Early models

The origins of contemporary modelling lie in nineteenth-century epidemiology⁷ and demography⁸ models. From the former there is a direct link to the model of malaria transmission developed by Ross at the beginning of this century⁹⁻¹². His original work was of enormous significance not only in epidemiology but also in the broader field discussed here because he clearly established the critical implications of the non-linearities in the system for control strategies (D. J. Bradley, inaugural lecture at the London School of Hygiene and Tropical Medicine). The model demonstrated that malaria could theoretically be eradicated without the need to eradicate the mosquito population as had previously been supposed: there was a critical threshold below which transmission of the malaria parasite ceased.

The nineteenth-century demographic models provided the basis for early theoretical studies of harvesting strategies. In the 1930s Graham¹³ and Hjort *et al.*¹⁴ independently drew attention to the management implications of the logistic equation as a model of population growth:

$$dx/dt = F(x) = rx(1 - x/K) \quad (1)$$

where r is proportional growth rate and K is the asymptotic stable equilibrium, traditionally referred to as the carrying capacity of the population environment. For this model the maximum sustainable yield (MSY) of, say, a fish population is obtainable by harvesting at a level $x = K/2$.

In subsequent decades few useful extensions were made to this basic work; it was not until the 1950s that progress was made with the development of a new generation of malaria and fish harvesting models and the first attempts to model agricultural and forest pests.

Large scale modelling

At the same time many ecologists became interested in the ability of modern computers and computer languages to mimic in detail

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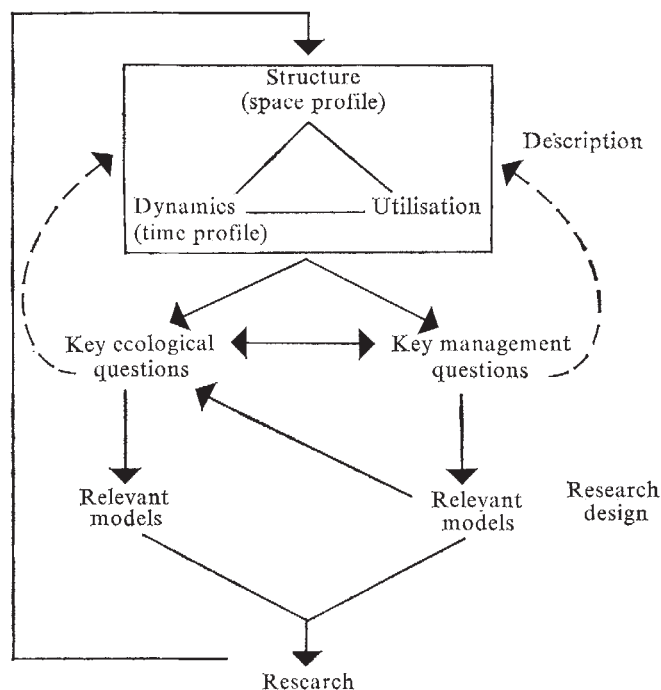


Fig. 1 A suggested procedure for applied ecosystem analysis, designed for a multidisciplinary team working on a continuing seminar basis. The procedure although flexible, is intended to ensure that ecological and management issues are raised simultaneously³⁶.

the dynamic and structural complexity of ecological systems¹⁵⁻¹⁹. This interest played an important part in the evolution of the International Biological Programme²⁰⁻²³ and in several of the IBP biome studies a central role was given to large scale ecosystem models.

A common approach to ecosystem modelling, both in the IBP and elsewhere has been to define ecosystems in terms of series of compartments—producers, consumers and decomposers, and an abiotic compartment—linked by equations describing energy (carbon) flows and materials cycling. The resulting models are essentially mechanistic, the hope being that the sum effect of the modelled interactions between and within compartments is sufficient to provide predictions of changes in the ecosystem, particularly the biomass of its constituent species, over relatively short periods.

A final assessment of the biome models would be premature now, since with some exceptions²⁴⁻²⁷ the relevant documentation is unpublished. There is no doubt that the models have conferred a better understanding of the complexity of ecosystem structure and dynamics, particularly on those engaged in the construction of the models^{21,28}. But so far there is little evidence that they have been useful in answering specific management questions²⁹. More successful in this respect have been aquatic ecosystem models which have taken both biotic and water quality into consideration, using mass balance equations^{30,31}. These can be used, for example, to assess the effects of the siting and quality of waste water discharges on rivers, lakes and other bodies of water. In general, though, while energy and material budgeting provides a convenient basis for linking ecosystem components the resulting models cannot easily be used by policy makers and managers whose prime concerns are the monetary and social outcomes of manipulating ecosystems. In practice, management issues are best served by purpose-built models³²⁻³⁴.

The alternative approach is not to attempt, at least at first, to match complex ecosystems with complex models³⁵, but to assume that their essential structure and behaviour can be understood and predicted through a limited number of key processes and that management problems can similarly be solved by altering a few key decisions³⁶. The task of the applied ecologist then becomes that of identifying the key questions and of choosing such appropriate mathematical models as will provide the answers (Fig. 1).

This approach, with its explicit avoidance of large-scale models, carries no guarantee that important features of the ecosystem will not be missed. However, in the absence of holistic models which are both sufficiently realistic and tractable it is probably more fruitful to concentrate on smaller-scale models designed to answer specific chosen questions, relying on multi-disciplinary interaction and the use of various graphic and flow diagram techniques to provide a holistic framework for the question posing. There is a greater likelihood of building models which are both practical and likely to contribute significantly to the emerging body of theory.

The remainder of this review is devoted, with some exceptions, to models which are based either implicitly or explicitly on these assumptions.

Vector borne disease

The new malaria model^{37,38} produced by Macdonald in the 1950s was based on that of Ross but was more biologically realistic, for example incorporating the possibility of superinfection. Central to the model is a function for the rate of malaria reproduction, for stable populations and static epidemiological conditions, of the form

$$Z_0 = bC/r \quad (2)$$

where Z_0 is the basic reproduction rate of malaria, or the number of infections distributed in a community as a direct result of the presence of a single, primary non-immune case, b is a measure of the probability of a human becoming infected if he is bitten by a mosquito with sporozoites in its glands, r is the recovery rate in man and C , the vectorial capacity

$$C = \frac{ma^2p^n}{\ln(1/p)} \quad (3)$$

where m is a measure of the density, a the man biting rate and p the daily survival rate of the mosquito population, and n the length of the parasite cycle in the mosquito.

Formulated like that the model is an elegant and powerful didactic tool with important implications for the broad strategies of malaria control. Simple inspection of the model reveals that strategies of killing larval mosquitoes, or administering prophylactic or curative drugs have a linear effect on the malaria reproduction rate, through their respective impacts on the terms m , b and r . However the strategy of insecticide spraying against the adult mosquitoes not only lowers m but also reduces p , and since this term enters the function non-linearly, small reductions in p result in disproportionately large reductions in the reproduction rate. This insight has been important in justifying the reliance on adulticide spraying as a principal malaria control strategy since World War II.

More recently, however, an attempt to use Macdonald's model to guide a local field control project has met with failure³⁹. Simulation studies⁴⁰, were used to prescribe the specific tactics for a strategy of chemotherapy combined with DDT spraying, aimed at interruption of malaria transmission in the Kankiya district of North-Central Nigeria, where earlier campaigns had failed. Part of the subsequent failure of the project was due to operational problems; the insecticide spraying was seriously delayed and proved ineffective against the particular vector population, *Anopheles gambiae*. It also became clear, however, that there were fundamental problems in the formulation and application of the model. The problems, moreover, seem similar to those encountered when attempts have been made to use models for tactical decisions in other fields such as fish harvesting or agricultural and forest pest control.

First, Macdonald's model is too aggregated and thus ignores both spatial heterogeneity and the important population subdivisions of age and state: the parameter b , for example, subsumes a complex of different biological processes including the host immune response which results in different immunological classes in the human population. Second, even when parameters seem

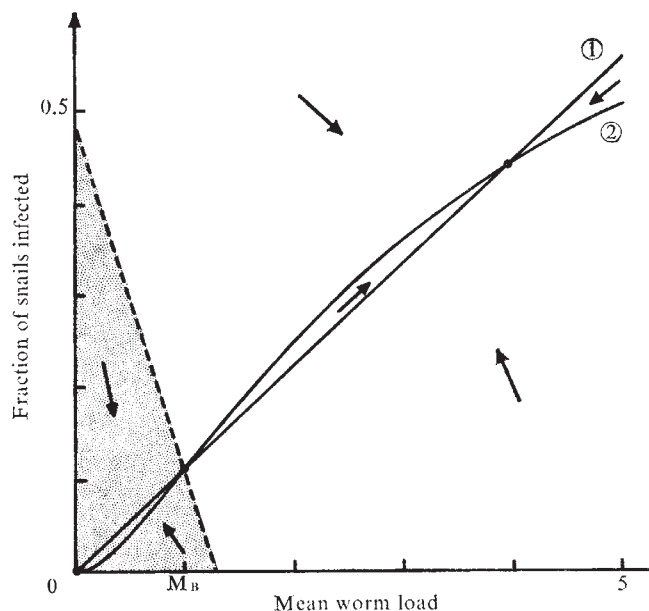


Fig. 2 Dynamics of Schistosome transmission based on elaboration of Macdonald's model. The two isoclines for stationary mean worm load (1) and fraction of snails infected (2) intersect at two stable points and at an unstable 'breakpoint' M_B below which the disease goes to extinction⁴⁷.

valid and well defined, the survival rate p for example, they are often difficult to measure with accuracy in the field. Third, being essentially deterministic and designed for stable populations, the model is not appropriate for the specific local conditions in Nigeria which are characterised by a seasonal and explosive epidemics.

Reviewing this episode Najera³⁹ acknowledges the value of Macdonald's model in its time but argues for the development of a third generation of malaria models, reflecting greater biological realism and the change from the objective of malaria eradication to malaria control. A deterministic model developed by Dietz *et al.*⁴¹ which recognises seven distinct epidemiological classes in the human populations is a step in this direction. As with earlier models a threshold vectorial capacity is specified below which malaria cannot maintain itself, but unlike Macdonald's model, where 100% endemicity is rapidly reached once the threshold is passed, the immune mechanism now realistically regulates the endemic level, and the model thus provides a truer measure of control in terms of the reduction in endemicity. Partial field tests have been applied to the model and it is currently being used to evaluate control strategies.

Schistosomiasis has recently received increasing attention from mathematical modellers⁴²⁻⁵². The disease is considerably more complex and much of the modelling effort has been focused on specific components of the disease cycle. There have, however, also been significant attempts to uncover the non-linearities in the overall life cycle of the disease which involves the transmission of the schistosome worms between the human and snail host. The classic model is again due to Macdonald⁵¹ who demonstrated the existence of a theoretical threshold or 'breakpoint' in the mean worm load in man, below which the disease goes to extinction (Fig. 2). The model further revealed that the threshold condition is more sensitive to changes in the probability of transmission from snail to man than man to snail and led him to suggest that the strategic priority should go to ensuring safe water supplies rather than improved sanitation. Macdonald's results have been modified by several subsequent workers: Bradley and May^{47,52}, for example, have shown that the assumption of a non-random distribution of worms in man significantly alters the threshold criteria.

May also observes that further non-linearities are likely to be introduced in the schistosome model once the economic costs of control are considered. So far no-one has attempted to combine economic and ecological features in a vector borne disease model,

although cost-benefit estimates have been derived for a number of situations⁵³⁻⁵⁶. Such estimates tend to be unsatisfactory because of the difficulty of obtaining realistic measures of benefits which in many cases are intrinsically unquantifiable. Loss of earning capacity as a result of morbidity or mortality can be calculated but there remains the problem of translating this loss into actual benefits following control, given the prevailing pattern of high unemployment and underemployment in the less developed countries. For the present it is more important to incorporate costs alone into epidemiological models, in particular so as to illuminate the changing cost pattern of a control or eradication campaign as it nears its objectives. The recent collapse of several malaria control campaigns suggests insufficient understanding of how maintenance costs and operations are determined by the epidemiological features of low endemicity. A related issue is the interaction of control campaigns for different diseases and the question of when it is 'safe' to switch budget priorities from one control campaign to another.

Harvesting

Policy models which integrate ecological and economic factors have been most successfully developed for fishery harvesting⁵⁷. This is partly due to the ease with which the basic logistic model used to describe fish population growth can be manipulated and extended⁵⁸⁻⁶⁰. Despite, or perhaps because of, its simple assumptions it has proved a powerful tool for illuminating fishery policy issues. The success of the 1946 Overfishing Convention has been attributed in part to Graham's forceful arguments based on the model⁶¹.

In the 1950s Schaefer⁶² incorporated fishing effort into the basic equation

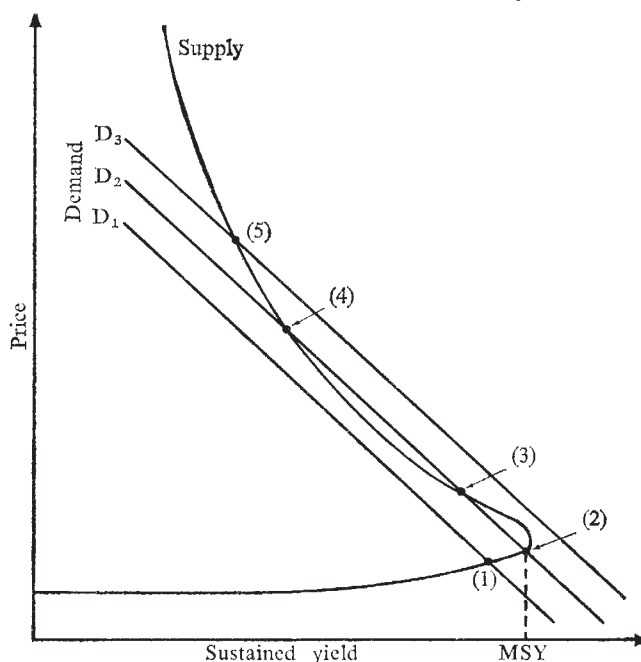
$$dx/dt = rx(1 - x/K) - qEx \quad (4)$$

where E is effort (for example vessel-days per unit time) and q the catchability coefficient. Gordon⁶³ then completed the economic formulation

$$\pi(x, E) = pqEx - cE \quad (5)$$

where $\pi(x, E)$ is net economic revenue, p and c are unit price and

Fig. 3 Instability of supply and demand in the open access fishery as revealed by the Schaefer model. For demand curve D_2 the equilibrium is at (2) or (4) depending on the starting population. However, a slight demand shift to D_3 results in inevitable overfishing (5)⁵⁷.



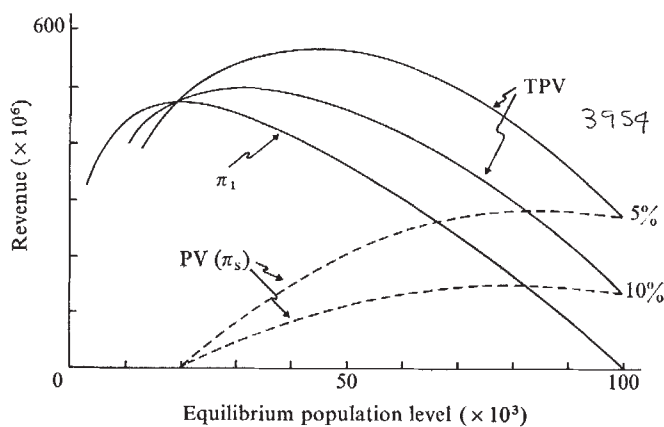


Fig. 4 Present value curves for blue whale harvesting at 5% and 10% discount rates. The total present value (TPV) is the sum of the net depletion revenue (π_1) obtained from depleting the initial stock (100,000) to the sustained yield level, and the present value of that sustained yield ($PV(\pi_s)$). Using a simple Schaefer model the MSY would be at 75,000 whales equilibrium population⁷¹.

cost, and used the model to show that in the open access fishery uncontrolled effort leads to an equilibrium at which total revenue equals total cost, so that the economic rent is completely dissipated. More recently several workers have extended his analysis to incorporate questions of supply and demand. For the open access fishery the supply curve for sustained yield is of the form

$$Y = rc(1 - c/pK)/p \quad (6)$$

High prices thus lead to biological overfishing. If the elasticity of demand is also taken into account the instability of the system becomes even more apparent (Fig. 3)^{57,64,65}.

The Schaefer model has played an important part in attempts to introduce some degree of regulation in otherwise open access fisheries. It provided, for example, one argument which led to the 1966 ban on blue whale harvesting^{66,67} and, in modified form, is currently used in calculating maximum sustainable yields (MSYs) and catch limits for all currently exploited whale stocks.

In the last two years, however, a number of criticisms have been levelled at fishery policy based on setting MSYs and catch quotas in this fashion¹²². One criticism stems from the deterministic nature of the models used. Beddington and May⁶⁹ demonstrate that in a randomly fluctuating environment the relative variability in population magnitude and hence yield increases systematically as harvesting effort increases. Moreover this effect is more pronounced when management is based on constant catch quotas rather than on a direct regulation of effort.

A more fundamental criticism is that the economic analysis ignores the important capital component in fishery management. As Clark and Munro^{57,70} forcefully point out a fishery is a capital asset and by definition its value is equal to the present value (PV) of the net future revenues it is expected to yield. In practice the commercial fishery aims not for an MSY but to maximise

$$PV = \int_0^{\infty} e^{-\delta t} - \pi(x, E) dt \quad (7)$$

where δ is the continuous discount rate per annum.

Clark has illustrated the practical consequences of this assumption in terms of blue whale harvesting⁷¹, where the maximum total present value is obtained at a stock level well below the MSY derived from a simple Schaefer model (Fig. 4). This result arises because the intrinsic rate of increase, r , of whale populations is of the order of 5–10%, which is similar to or often less than prevailing commercial discount rates. In many commercial fisheries the biometric growth rate δ/r is less than unity and the MSY is a sensible first approximation to a management goal but where δ

exceeds r , conservation can only be achieved by decisions which, in effect, replace the high commercial discount rate by a politically derived social rate.

As a practical management tool the Schaefer model is also too dependent on the availability of a long run of catch data. It may be the only approach where age grading of fish is impossible but for the majority of commercial fish an age class model is feasible and indeed essential if the detailed population dynamics, and in particular any non-linear stock-recruitment phenomena, are allowed for adequately. The first significant advance in this direction was the Beverton and Holt model⁷² which gives the annual biomass yield Y in equilibrium for a population consisting of cohorts of all ages

$$Y = R \exp(F t_{\mu}) \int_{t_{\mu}}^{\infty} \exp(-(M+F)t) w(t) dt \quad (8)$$

where R is annual recruitment, F and M the fishing and natural mortality coefficients, $w(t)$ the weight which is usually computed by the von Bertalanffy function and μ is the mesh size, so that t_{μ} is the age when fish are first caught. The model can thus be used to prescribe management strategies in terms of fishing mortality and mesh size. Its main application has been for North Sea demersal fish stocks. Beverton and Holt recommended a mesh size of the order 110 mm for plaice and haddock with a 80–90 mm mesh to ensure survival of sole and whiting, and by the early 1960s minimum mesh sizes of that order were in operation in the North Sea⁶⁶.

More recently, the Leslie matrix formulation^{73–76} has been used to represent the age structure of fish and game populations subject to harvesting

$$\mathbf{x}_{i+1} = \mathbf{A} \mathbf{D} \mathbf{x}_i \quad (9)$$

the matrix $\mathbf{A}$ having elements

$$a_{ij} = \begin{cases} f_j & \text{for } i = 1 \\ p_j & \text{for } i = j+1 \\ 0 & \text{otherwise} \end{cases}$$

where f_j and p_j are the age specific fecundity and natural survival. The diagonal matrix $\mathbf{D}$ with elements θ_i is the proportion of the i th age class surviving exploitation. The model has the merit of being conceptually simple and intelligible to non-mathematical biologists, although accurate field measurement of the necessary age specific parameters may present difficulties. Beddington⁷⁸ has, however, obtained reasonable estimates for red deer populations in Scotland. The harvest at equilibrium is given by

$$q = \sum_i (1 - \theta_i) n_i \quad (10)$$

and this is inserted in a Cobb–Douglas production function for the yearly crop

$$P = q X_3 = \beta X_1^{x_1} X_2^{x_2} X_3^{x_3} \quad (11)$$

where the X s are the number of stalkers, the area of the estate and the deer population respectively. Given the pattern of deer forest ownership in Scotland the estate size is not easily adjusted but the model can be used to provide a guide to the most profitable labour input.

Models such as the Beverton and Holt and Leslie matrix together with a variety of simulation models developed in the last few years provide the beginnings of tactical harvesting models, but there are many formidable problems to be overcome. In particular there are practical and theoretical difficulties in obtaining dynamic optimisation solutions to age structure models. These problems will be compounded when attempts are made to produce tactical versions from the few existing policy orientated models for multispecies harvesting and when, eventually, harvesting models encompass the whole ecosystem^{78,79}.

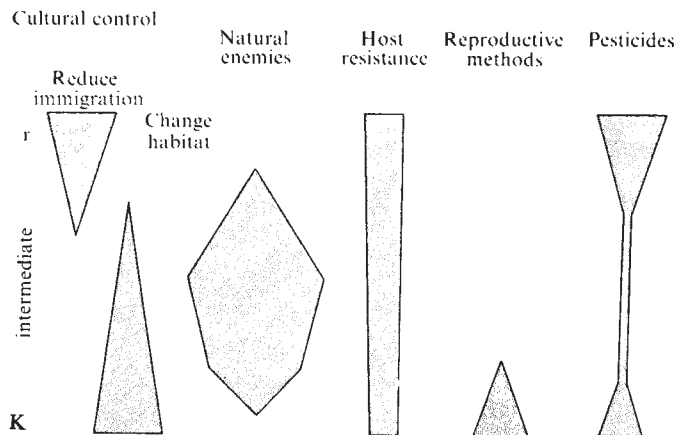


Fig. 5 Appropriate control strategies for pests categorised in terms of the r - K continuum⁸⁸.

Pest control

A further critical problem facing fishery ecologists is that of estimating parameters, in particular for density dependent processes^{61,80}. The theoretical consequences of different functional forms of these processes have been understood for some time but there are very considerable difficulties in obtaining accurate data. Insect populations, by contrast, can be more easily observed and experimented upon, so that considerably more is known about their population dynamics. This is particularly true of the relationships between insects and their predators and parasites, for which there is a range of mathematical models that have been well tested against laboratory and, less well tested against field data⁸¹⁻⁸⁵.

Surprisingly, however, there has been little effort to use these models to formulate strategies for pest control. A notable exception is Hassell and May's⁸⁶ analyses of the characteristics of insect parasitoids as agents of biological control. In a model which explicitly deals with spatial heterogeneity, population change for the host pests (X) and the parasitoids (P) is given by

$$X_{t+1} = \lambda X_t \sum_{i=1}^n [\alpha_i \exp(-a(c\alpha_i^\mu P_t)^{1-m})] \quad (12)$$

and

$$P_{t+1} = X_t - X_{t+1}/\lambda \quad (13)$$

where λ is the net rate of host increase, the $\{\alpha_i\}$ set defines the host distribution and μ , a and m are measures of aggregation, searching efficiency and mutual interference in the parasitoids. In general, high levels of a lead to low equilibria and high levels of m and μ to stability in the host population. Since highly specific parasitoids tend to show these features more markedly than polyphagous ones, it is argued that specific natural enemies are preferable as biological control agents where, for example in perennial orchards, equilibrium populations can be more readily maintained.

Recently, Conway⁸⁷ and Southwood⁸⁸ have attempted to provide a stronger link between theoretical ecological models and practical pest control, by applying the r - K selection continuum to the choice of control strategies. They distinguish at one extreme, r -pests such as the desert locust, characterised by high rates of dispersal and highly damaging population explosions, and at the other extreme K -pests such as the codling moth, with lower rates of increase, regulated by competition and whose status as pests is achieved more because of the character of the damage they cause. In between lies a large category of pests normally regulated at various levels by parasites and predators. Simple models^{89,90} have been produced for the different categories and used to prescribe general rules for control (Fig. 5). Although these notions are still somewhat crude and oversimplified they provide the beginnings of a fairly rigorous strategy analysis for pest control.

A good example of the intelligent application of simple models of this order is in pesticide resistance. Again, surprisingly, there have been no attempts until very recently⁹¹⁻⁹³ to model resistance in spite of its critical importance in modern pest control practice. Comins^{94,95} has, however, developed a deterministic Mendelian model which demonstrates, for example, the existence of a hysteresis effect in the presence of migration from untreated populations, the population jumping from high susceptibility to high resistance as migration drops below a critical level. Density dependence is included in the model in the form

$$X_{t+1} = \lambda X_t^{1-b} \quad (14)$$

where b is the density dependence coefficient. For perfect density dependence ($b = 1$) resistance time decreases with increasing kill but where the relationship is undercompensating ($0 < b < 1$) intermediate kills result in the fastest resistance. In general, for reasonable levels of density dependence it seems that resistance can be suppressed by using highly effective non-residual insecticides, strictly confined to the target population.

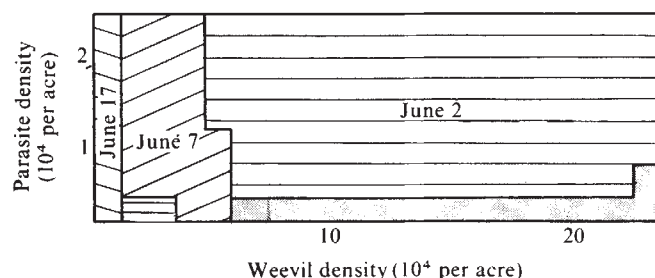
Simple density dependence models have also been used to explore the interrelationships between ecological and economic factors in pest control. In a study of the sugar cane froghopper, *Aeneolamia varia saccharina*, Conway *et al.*⁹⁶ show how the optimal spraying strategy is a function of the degree of density dependence between the different broods that the froghopper passes through in a season. In the absence of density dependence ($b = 0$) heavy spraying in the first brood is optimal but as the relationship goes from undercompensating to overcompensating ($1 < b < 2$) the spraying becomes progressively heavier in later broods. Models of this kind are not prescriptive but play an important part in determining which of a multitude of ecological factors merit further research before control decisions are made.

Dynamic programming⁹⁷ was used to arrive at the froghopper result and this technique has been used to derive optimal strategies for a number of pests^{91,92,98-101}. Its usefulness is restricted by the number of variables which can be included, but if this problem is resolved dynamic programming has a considerable potential as a tool for day-to-day management. As Shoemaker has shown⁹⁸ the results can be presented as simple decision rules in graphic form (Fig. 6), which allow an optimal strategy to be pursued even when the outcome of earlier decisions has not been as expected.

The problem of modelling age structure has also been tackled, as in harvesting models, by use of the Leslie matrix formulation^{96,102}. Recently Gutierrez, Wang *et al.*¹⁰³⁻¹⁰⁵ have used a continuous form, which is time and age dependent, to model both plant growth and the pest population on cotton and alfalfa. They seem to have been fairly successful in obtaining appropriate data but in general age class parameters are difficult to measure in many insect populations.

To date, most attempts at tactical models have taken the form of complex computer simulations. At worst they have suffered from the defects of the large-scale ecosystem models but there have been some notable exceptions, kept functional and useful because they have concentrated on the fairly circumscribed question of the optimal timing of pesticide application. Regev *et al.*⁹⁸ have developed such a model for the alfalfa weevil, *Hypera brun-*

Fig. 6 Optimal control strategies for the alfalfa weevil in terms of a standard insecticide spray (shaded area) and date of alfalfa harvesting¹⁰⁰.



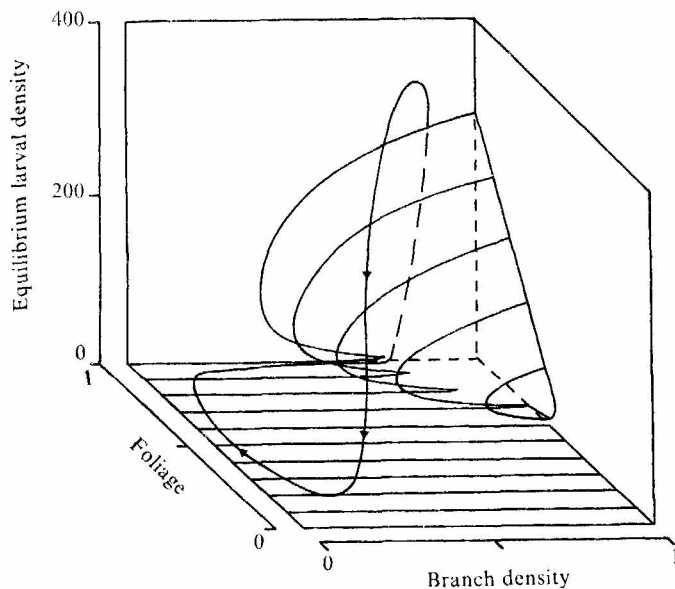


Fig. 7 Path of a single outbreak cycle of the spruce budworm in terms of living foliage per branch and different densities of branches per acre¹¹⁰.

neipennis, and demonstrated that higher profit would accrue from earlier spraying in the season. Kiritani and his colleagues¹⁰⁶⁻¹⁰⁹ have similarly used a simulation model of the dynamics of the green leafhopper *Nephotettix cincticeps*, and the *Lycosa* spiders which are its principal enemies to demonstrate the detrimental effects of BHC spraying which leads to destruction of the spiders and resurgence of the leafhopper. As a consequence the insecticide has been phased out from control campaigns in Japan.

Perhaps the most successful simulation model has been developed by Holling and his co-workers¹¹⁰ for the spruce budworm (*Choristoneura fumiferana*) in New Brunswick. At first a very complex model, it has been progressively bounded so that the essential dynamics of the system (Fig. 7) can be represented in terms of a limited number of variables relating to the pest, its natural enemies, the three main tree species, weather and, significantly, space. The final model reproduces the budworm dynamics for a 17,000 square mile region in terms of 265 discrete subdivisions. The model has been used to derive strategies of control under a variety of objectives, one set of outputs being in the form of graphical decision rules (compare Fig. 6) for spraying and logging obtained by a Markovian optimisation procedure.

Decision making

Underlying most policy models is the assumption that the farmer or trawler owner is a profit maximiser and hence is risk neutral; that is he values each successive increment in income in the same way. In practice, however, resource managers, whether at the individual, corporate or national level, exhibit a wide range of attitudes toward the outcomes of their decisions. Norton¹¹¹⁻¹¹³ has recently illustrated the problem in the context of pest control and demonstrated the value of Bayesian techniques in the relatively common situation where the farmer is risk averse; that is, he values initial increases in income far higher than later increases. For example Bayesian analysis of potato blight control in England with the objective of a long run average profit maximisation leads to schedule spraying in the Southwest and Fens and no spraying in the Southern and Northern Regions. Because of the high variance associated with no spraying, however, the risk averse farmer in the latter regions may decide to spray anyway. Bayesian analysis can be further usefully extended to assessments of the value of pest forecasting. It is often naively assumed that a forecast is always useful but in practice the value depends on critical threshold levels in the accuracy of the forecast.

Such decision making has eventually to be placed in the broader context of ecosystem and regional management, which is likely to have to resolve conflicting and often incommensurate objectives. The most promising of the multivariate decision techniques is multi-attribute utility analysis^{114,115}, which has the advantage of dealing with uncertainty in a rigorous manner and of directly involving the decision maker in the definition and weighting of objectives. In common with other techniques, however, it is essentially static in that it seeks to identify an optimal plan which is adhered to for the foreseeable future. But, experience shows that objectives change or hidden objectives become manifest and unsuspected and unforeseeable events arise to disturb the management plan. Walters¹¹⁶ argues that this problem is compounded by a tendency for a wrong policy or design decision to beget further wrong decisions when dramatic problems arise so that, in practice, there is a progressive foreclosure of options. These are issues which have been addressed in the spruce budworm¹¹⁰ and a related salmon study¹¹⁷⁻¹¹⁹. A general conclusion is that the main goal of such studies and the models they generate is not to solve an economic maximisation function but to create some kind of robust system which is resilient to all conceivable ecological, economic, social and political change^{120,121}.

No unified theory

It is very clear from this review that mathematical modelling in the fields of vector borne disease, harvesting and pest control has proceeded at different rates and in largely different directions, despite Watt's vision of a unified science of resource management. Yet, it is also clear that each field could well profit from the experience of the others. Policy models, of the kind developed for fishery management are urgently required in both pest and vector borne disease control. Equally, the notions and models of density dependence that are being elaborated for pest control could provide a more powerful basis for determining strategies for harvesting and disease vector control. There is also a growing, albeit halting experience, in each field of attempts to incorporate the discrete variables of age, state and space in tactical models and to develop appropriately dynamic optimisation techniques, which needs to be shared. From this kind of cross fertilisation a common theory and practice of applied ecology could begin to emerge.

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- Watt, K. E. F. *Ecology and Resource Management* (McGraw-Hill, New York, 1968).
- Conway, G. R. in *Insects: Studies in Population Management* (eds Geier, P. W., Clark, L. R., Anderson, D. J. & Nix, H. A.) 103-120 (Ecological Society of Australia, Canberra, 1973).
- Jeffers, J. N. R. (ed.) *Mathematical Models in Ecology* (Blackwell Scientific, Oxford, 1972).
- Patten, B. C. (ed.) *Systems Analysis and Simulation in Ecology 1 & 2* (Academic, New York, 1971 & 1972).
- Ruesink, W. G. *A. Rev. Ent.* **21**, 27-44 (1976).
- Van Dyne, G. M. & Abramsky, Z. in *Study of Agricultural Systems* (ed. Dalton, G. E.) 23-106 (Applied Science, London, 1975).
- Farr, W. J. *Soc. Sci.* **233**, 236 (1866).
- Verhulst, P. F. *Correspondance Mathematique et Physique* **10**, 113-121 (1838).
- Ross, R. *Report on the prevention of Malaria in Mauritius*, London (1908).
- Ross, R. *The Prevention of Malaria*, 2nd ed. London (1911).
- Ross, R. *Nature* **87**, 466-467 (1911).
- Ross, R. *Proc. R. Soc. A* **92**, 204-230 (1916).
- Graham, G. M. J. *Cons. perm. int. Explor. Mer.* **10**, 264-274 (1935).
- Hjort, J., Jahn, G. & Ottestad, P. *Hvalrad. Skr.* **7**, 92-127 (1933).
- Gorfinckel, D. in *Computers in Biomedical Research 2* (eds Stacey, R. W. & Waxman, B.) 205-216 (Academic, New York, 1965).
- Holling, C. S. *Mem. Entomol. Soc. Can.* **48**, 86 (1966).
- Jones, J. G. W. in *Rep. Grassld. Res. Inst.* 1965, 51-52 (1966).
- Van Dyne, G. M. *Oak Ridge Natl. Lab. Tech. Memo.* 3957, 40 (1966).
- Watt, K. E. F. (ed.) *Systems Analysis in Ecology* (Academic, New York, 1966).
- Neuhoff, J. M. in *Systems Analysis and Simulation in Ecology 3* (ed. Patten, B. C.) 7-12 (Academic, New York, 1975).
- O'Neill, R. V. in *Ecological Modelling in a Resource Management Framework* (ed. Russell, C. S.) 251-282 (Resources for the Future, Washington, 1975).
- Van Dyne, G. M. in *Mathematical Models in Ecology* (ed. Jeffers, J. N. R.) 111-172 (Blackwell Scientific, Oxford, 1972).
- Worthington, E. B. (ed.) *The Evolution of IBP* (Cambridge University, Cambridge, 1975).
- Park, R. A., Scavia, D. & Clesceri, N. L. in *Ecological Modelling in a Resource Management Framework* (ed. Russell, C. S.) 13-48 (Resources for the Future, Washington, 1975).
- Park, R. A. et al. *Simulation* **23**, 33 (1974).
- Patten, B. C. (ed.) *Systems Analysis and Simulation in Ecology 3 & 4* (Academic, New York, 1975 & 1976).
- Shugart, H. H., Goldstein, R. A., O'Neill, R. V. & Mankin, J. B. *Oecol. Plant.* **9**, 231 (1974).
- Woodmansee, R. G. *Critique and analysis of the grassland ecosystem model ELM 93* (US IBP Grassland Biome, Natural Resource Ecological Laboratory, Colorado State University, 1975).
- Watt, K. E. F. in *Systems Analysis and Simulation in Ecology 3* (ed. Patten, B. C.) 139-152 (Academic, New York, 1975).

- ⁴⁰ Chen, C. W. & Orlob, G. T. in *Systems Analysis and Simulation in Ecology* 3 (ed. Patten, B. C.) 476-588 (Academic, New York, 1975).
- ⁴¹ Kelly, R. A. in *Systems Analysis and Simulation in Ecology* 4 (ed. Patten, B. C.) 3-45 (Academic, New York, 1976).
- ⁴² Biswas, A. K. *Ecological Modelling* 1, 31-48 (1975).
- ⁴³ Holcomb Research Institute. *Environmental Modeling and Decision Making* (Praeger, New York, 1976).
- ⁴⁴ Mitchell, R., Mayer, R. A. & Downhower, J. *Science* 192, 859-865 (1976).
- ⁴⁵ Walker, K. E. F. *Simulation* 28, 1-3 (1977).
- ⁴⁶ Walker, B. H., Norton, G. A., Conway, G. R., Comins, H. N. & Birley, M. J. *appl. Ecol.* (in the press).
- ⁴⁷ Macdonald, G. *The Epidemiology and Control of Malaria* (Oxford University, London, 1957).
- ⁴⁸ Macdonald, G. *Publ. Hlth Rep., Wash.* 76, 753-764 (1961).
- ⁴⁹ Najera, J. A., *Bull. Wld Hlth Org.* 50, 449-457 (1974).
- ⁵⁰ Macdonald, G., Cuellar, C. B. & Foll, C. V. *Bull. Wld Hlth Org.* 38, 743-755 (1968).
- ⁵¹ Dietz, K., Molineaux, L. & Thomas, A. *Bull. Wld Hlth Org.* 50, 347-357 (1974).
- ⁵² Cohen, J. E. *Ann. Rev. Ecol. Syst.* (in the press).
- ⁵³ Cohen, J. E. in *Theoretical Ecology: Principles and Applications* (ed. May, R. M.) 237-256 (Blackwell Scientific, Oxford, 1976).
- ⁵⁴ Fine, P. E. M. (ed.) *Mathematical Models of Schistosomiasis* (Edna McConnell Clark Foundation, New York, 1976).
- ⁵⁵ Hairston, N. G. in *Epidemiology and Control of Schistosomiasis (Bilharzia)* (ed. Ansari, N.) (Basel, University Park, Baltimore, 1973).
- ⁵⁶ Lee, K. L. & Lewis, E. R. *IEEE Trans. Biomed. Eng.* BME-23, 225-233 (1976).
- ⁵⁷ May, R. M. *Math. Biosci.* (in the press).
- ⁵⁸ Nasell, I. *Theor. Pop. Biol.* 10, 47-69 (1976).
- ⁵⁹ Nasell, I. *Theor. Pop. Biol.* 10, 133-144 (1976).
- ⁶⁰ Nasell, I. & Hirsch, W. M. *Commun. pure appl. Math.* 26, 395-453 (1973).
- ⁶¹ Macdonald, G. *Trans. R. Soc. trop. Med. Hyg.* 59, 489-506 (1965).
- ⁶² Bradley, D. J. & May, R. M. *Proc. R. Soc. trop. Med. Hyg.* (in the press).
- ⁶³ Fenwick, A. *Bull. Wld Hlth Org.* 47, 573-578 (1972).
- ⁶⁴ Fenwick, A. & Fienschou, B. H. *Bull. Wld Hlth Org.* 47, 567-572 (1972).
- ⁶⁵ Mears, L. A. *Economic Project Evaluation with Philippine Cases* (University of the Philippines, Manila, 1969).
- ⁶⁶ Wright, W. H. *Bull. Wld Hlth Org.* 47, 559-566 (1972).
- ⁶⁷ Clark, C. W. *Mathematical Bioeconomics: The Optimal Management of Renewable Resources* (Wiley-Interscience, New York, 1976).
- ⁶⁸ Christy, F. T. *Alternative Arrangements for Marine Fisheries: An Overview* (Resources for the Future, Washington DC, 1973).
- ⁶⁹ Graham, M. *Cons. int. L'Explor. Mer. Rapp. P.-v. Reun.* 132, 72-78 (1952).
- ⁷⁰ Gulland, J. A. *The Management of Marine Fisheries* (University of Washington, Seattle, 1974).
- ⁷¹ Cushing, D. *Marine Ecology and Fisheries* (Cambridge University, London, 1975).
- ⁷² Schaefer, M. B. J. *Fish. Res. Bd Can.* 14, 669-681 (1957).
- ⁷³ Gordon, R. L. J. *Polit. Econ.* 62, 124-142 (1954).
- ⁷⁴ Anderson, L. G. J. *Fish. Res. Bd Can.* 30, 509-518 (1973).
- ⁷⁵ Copes, P. *Manchester School of Social and Economic Studies* 40, 145-163 (1972).
- ⁷⁶ Cushing, D. *Fisheries Resources of the Sea and their Management* (Oxford University, Oxford, 1975).
- ⁷⁷ Chapman, D. G., Allen, K. R. & Holt, S. J. *Rep. int. Commn Whal.* 14, 32-106 (1964).
- ⁷⁸ Gambell, R. *Appl. Biol.* 1, 247-343 (1976).
- ⁷⁹ Beddington, J. R. & May, R. M. *Science* (in the press).
- ⁸⁰ Clark, C. W. & Munro, G. R. *J. Envir. Econ. Management* 2, 92-106 (1975).
- ⁸¹ Clark, C. W. *Advisory Committee on Marine Resources Research ACMRR, MM SC 65* (F.A.O., London, 1976).
- ⁸² Beverton, R. J. H. & Holt, S. J. *On the Dynamics of Exploited Fish Populations* (Min. Ag. Fish & Food Fisheries, Investigation Series 219), London, 1957).
- ⁸³ Beddington, J. R. & Taylor, D. B. *Biometrics* 29, 801-809 (1973).
- ⁸⁴ Leslie, P. H. *Biometrics* 35, 213-245 (1948).
- ⁸⁵ Reed, W. J. *Math. Biosci.* 22, 313-337 (1974).
- ⁸⁶ Usher, M. B. in *Mathematical Models in Ecology* (ed. Jeffers, J. N. R.) 29-60 (Blackwell Scientific, Oxford, 1972).
- ⁸⁷ Beddington, J. R. *J. Envir. Management* 3, 91-103 (1975).
- ⁸⁸ Walsh, J. J. in *The Ecology of the Seas* (eds Cushing, D. H. & Walsh, J. J.) 388-407 (Blackwell Scientific, Oxford, 1976).
- ⁸⁹ Steele, J. H. *The Structure of Marine Ecosystems* (Harvard University, Cambridge, 1974).
- ⁹⁰ Parrish, B. B. (ed.) *Fish Stocks and Recruitment Rapp. P.-v. Reun. Cons. int. Explor. Mer* 164 (1973).
- ⁹¹ Beddington, J. R., Free, C. A. & Lawton, J. H. *J. anim. Ecol.* 45, 791-816 (1976).
- ⁹² Gilbert, N., Gutierrez, A. P., Frazer, B. D. & Jones, R. E. *Ecological Relationships* (Freeman, San Francisco, 1976).
- ⁹³ Hassell, M. P. in *Theoretical Ecology: Principles and Applications* (ed. May, R. M.) 71-93 (Blackwell Scientific, Oxford, 1976).
- ⁹⁴ Murdoch, W. W. & Oaten, A. *Adv. Ecol. Res.* 9, 2-131 (1975).
- ⁹⁵ Royama, T. *Res. Popul. Ecol. Suppl.* 1, 1-91 (1971).
- ⁹⁶ Hassell, M. P. & May, R. M. *J. anim. Ecol.* 42, 693-726 (1973).
- ⁹⁷ Conway, G. R. in *Theoretical Ecology: Principles and Applications* (ed. May, R. M.) 257-281 (Blackwell Scientific, Oxford, 1976).
- ⁹⁸ Southwood, T. R. E. in *Origins of Pest, Parasite, Disease and Weed Problems* (eds Cherrett, J. M. & Sagar, G. R.) 35-54 (Blackwell Scientific, Oxford, 1977).
- ⁹⁹ Southwood, T. R. E., May, R. M., Hassell, M. P. & Conway, G. R. *Am. Nat.* 108, 791-804 (1970).
- ¹⁰⁰ Southwood, T. R. E. & Comins, H. N. *J. anim. Ecol.* 45, 949-965 (1976).
- ¹⁰¹ Hueth, D. & Regev, U. *Am. J. agr. Econ.* 56, 543-552 (1974).
- ¹⁰² Regev, U., Shalit, H. & Gutierrez, A. P. in *Proc. IIASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IIASA, Laxenburg, in the press).
- ¹⁰³ Taylor, C. R. & Headley, C. *Ent.* 107, 237-242 (1975).
- ¹⁰⁴ Comins, H. N. *J. theor. Biol.* 64, 177-197 (1977).
- ¹⁰⁵ Comins, H. N. *J. theor. Biol.* 65, 399-420 (1977).
- ¹⁰⁶ Conway, G. R., Norton, G. A., Small, N. J. & King, A. B. S. in *Study of Agricultural Systems* (ed. Dalton, G. E.) 193-229 (Applied Science, London, 1975).
- ¹⁰⁷ Bellman, R. *Dynamic Programming* (Princeton Univ., Princeton, 1957).
- ¹⁰⁸ Regev, U., Gutierrez, A. P. & Feder, G. *Am. J. agr. Econ.* 58, 188-195 (1900).
- ¹⁰⁹ Shoemaker, C. A. *Math. Biosci.* 18, 1-22 (1973).
- ¹¹⁰ Shoemaker, C. A. in *Proc. IIASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IIASA, Laxenburg, in the press).
- ¹¹¹ Watt, K. E. F. *Can. Ent.* 95, 525-636 (1963).
- ¹¹² Conway, G. R. in *Proc. XV International Congress of Entomology* 541-552 (Washington, DC, 1976).
- ¹¹³ Gutierrez, A. P., DeMichele, D. W. & Wang, Y. in *Proc. XV International Congress of Entomology* 553-559 (Washington, DC, 1976).
- ¹¹⁴ Wang, Y., Gutierrez, A. P. & Oster, G. *Can. Ent.* (in the press).
- ¹¹⁵ Gutierrez, A. P. in *Proc. IIASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IIASA, Laxenburg, in the press).
- ¹¹⁶ Kiritani, K. in *Proc. XV International Congress of Entomology* 591-598 (Washington, DC, 1976).
- ¹¹⁷ Kiritani, K. in *Proc. IIASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IIASA, Laxenburg, in the press).
- ¹¹⁸ Sasaba, T. & Kiritani, K. *Res. Popul. Ecol.* 16, 231-244 (1975).
- ¹¹⁹ Sasaba, T., Kiritani, K. & Urabe, T. *Res. Popul. Ecol.* 15, 9-22 (1973).
- ¹²⁰ Holling, C. S., Jones, D. D. & Clark, W. C. in *Proc. IIASA Pest Management Conference* (eds Norton, G. A. & Holling, C. S.) (IIASA, Laxenburg, in the press).
- ¹²¹ Norton, G. A. *Ann. appl. Biol.* 84, 444-447 (1976).
- ¹²² Norton, G. A. *Agroecosystems* 3, 27-44 (1976).
- ¹²³ Norton, G. A. & Conway, G. R. in *Origin of Pest, Parasite, Disease and Weed Problems* (eds Cherrett, J. M. & Sagar, G. R.) 205-226 (Blackwell Scientific, Oxford, 1977).
- ¹²⁴ Keeney, R. L. *IASA Research Memorandum* 75-43 (1975).
- ¹²⁵ Keeney, R. L., Wood, E. F., David, L. & Csontos, K. *IASA Collaborative Publication* 76-3 (1976).
- ¹²⁶ Walters, C. J. *IASA Research Report* 75-12 (1975).
- ¹²⁷ Hilborn, R. & Peterman, R. M. in *Management for People* (ed. Ellis, D. V.) 68-98 (University of Victoria, British Columbia, 1977).
- ¹²⁸ Hilborn, R. & Walters, C. J. *J. Fish Res. Bd Can.* 34, 64-72 (1977).
- ¹²⁹ Keeney, R. L. *J. Fish Res. Bd Can.* 34, 49-63 (1977).
- ¹³⁰ Holling, C. S. *Ann. Rev. Ecol. Syst.* 4, 1-24 (1973).
- ¹³¹ Holling, C. S. *Proc. Conf. Future Energy Strategies* (Oak Ridge Associated Universities, in the press).
- ¹³² Larkin, P. A. *Trans. Am. Fish. Soc.* 106, 1-11 (1977).

articles

Ocean-floor magnetotelluric sounding over North Central Pacific

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Observations on the sea floor of naturally occurring electromagnetic signals reveal information on the deep structure of the Earth. Recent magnetotelluric soundings on the North Central Pacific have produced conductivity measurements which may relate to the partially molten zone or asthenosphere on which the well lubricated oceanic plates slide.

OBSERVATIONS over a wide range of frequencies of slowly fluctuating natural electric and magnetic fields at the Earth's surface can be used to infer the distribution of electrical conductivity in the Earth's crust and mantle^{1,2}. Because of the

strong dependence of the electrical conductivity of terrestrial materials on temperature and composition³ the magnetotelluric (MT) technique is now accepted as a valid and attractive method of geophysical investigation on land⁴⁻⁸. Providing that electric and magnetic fluctuating fields can be recorded on the sea floor, the MT method is also applicable to indirect exploration of the oceanic basement although high frequency shielding by the ocean tends to mask the influence of crustal layers⁹. A recent MT sounding on the North Central Pacific suggests a rapid initial conductivity increase downward through lithosphere and upper mantle, weakening sharply and probably reversing trend around 180 ± 40 km. The resulting high conductivity layer may relate to the partially molten zone

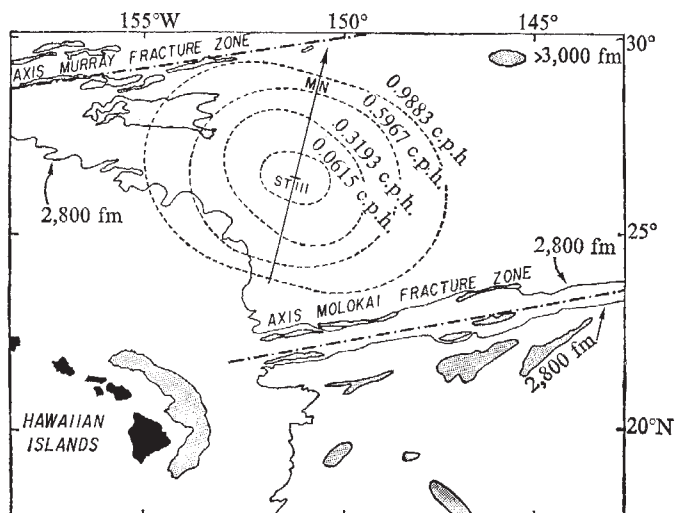


Fig. 1 Location of the observation station ($26^{\circ}35.9'N$, $151^{\circ}09.8'W$). The four elongated figures represent the ratio of the electric field to the magnetic field at right angle for four frequencies and with respect to the azimuth of the electric field. Important bathymetric features are also shown.

or asthenosphere on which the well-lubricated oceanic plate is allowed to slide.

The first suggestion of MT exploration at sea is in Cagniard's original paper², together with a symbolic diagram and the assertion that performing MT soundings on the ocean floor presents no special difficulty. In spite of Cagniard's optimism, as well as the actual simplicity inherent in the principles of the MT method, its application to submarine geophysics has progressed extremely slowly, probably because of the natural hostility of the oceanic environment and the remoteness of the deep sea floor.

The first MT success at sea was scored^{10,11} with a unidirectional MT sounding 600 km off Central California in 1965. In spite of many limitations, this contributed important confirmatory evidence of the considerable difference between continental and oceanic lithospheres, thus enhancing interest in the MT method in offshore geophysics. Newer data¹² collected in 1973 in the West Central Atlantic are being analysed. Nevertheless, the anticipated contribution of sea floor MT to submarine geology and geophysics has remained unproven until very recently.

In autumn, 1976, a new MT sounding was performed in the North Central Pacific with redundant equipment much superior to previously available instrumentation, see Fig. 1. As a result, the electric to magnetic field coherence and concurrently the resolution in characterisation of conductivity structure, improved enough to produce a meaningful structural picture of the local sea floor basement which seems compatible with other accepted knowledge.

Fundamental to this recent advance was the development of instruments with extremely low drift and noise, yet with extremely high resolution ($10^{-7} V m^{-1}$ and $0.2 nT$). Electrode switching by means of a salt bridge chopper^{9,13} permitted essentially total rejection of electrode noise. (The natural signal of the optimum silver-silver chloride electrodes¹³ is typically 10^3 times larger than the signal to be recorded.) Conceptually, the magnetic sensors borrow from high grade fibre and magnet technology already optimised in the time of classical physics and from the most recent advances in modern optometrics.

Recording MT fluctuations

The first 2 weeks of a 3 week redundant recording cross coverage by two electric field recorders and two magnetometers are plotted on Fig. 2. **H** is the magnetic variation to the magnetic North, **D** to the magnetic East, and **Z** downward; **E_x** and **E_y**

are the horizontal electric field components to the magnetic North and East, respectively.

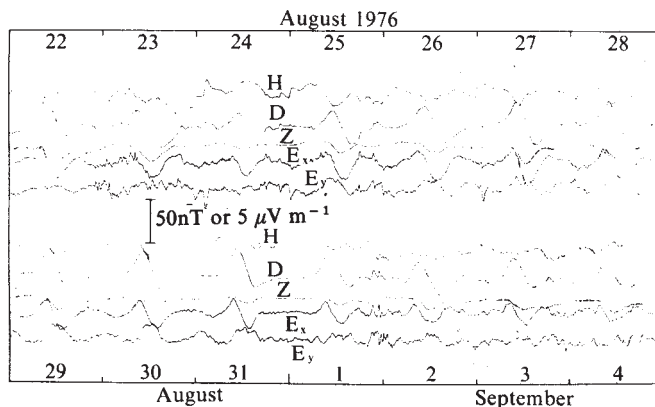
If Fig. 2 were compared with similar records covering a long period of time the following would be noticed. The daily modulation of the various fields is smooth and regular, particularly with respect to component **D**, although high frequency signals are often present. This relatively quiet behaviour is consistent with the time of collection of data with respect to the 11-yr sunspot and solar storm cycle which had reached its low point. The time reference in the above plot is Hawaii Standard Time (HST). Local noon at our station is therefore slightly early; the maximum descending slope of **D** at midday is slightly ahead with respect to noon, and the difference is consistent with the station longitude.

Although magnetic field disturbances (originated in the ionosphere and beyond, and controlled by fluctuations in the solar wind) as well as their electric field counterpart are numerous on the record, their amplitude is moderate to low, particularly with respect to the higher frequencies: in addition to the inherent low activity characteristic of the present solar cycle state, the shielding of the ocean greatly reduces such fluctuations above a few c.p.h. and essentially suppress them above 15 to 20 c.p.h. Note that most intense electric fields occur when the rate of change of the magnetic field is highest, a fact well exemplified by the rapid excursion of **H** early on 24 and on 27 August and the corresponding large electric spikes in **E_y**, at right angle to **H**.

The power spectra of the four components, **H**, **D**, **E_x**, **E_y**, have been calculated using a fast Fourier transform and band averaging as shown in Fig. 3; note that both spectra and frequency scales are logarithmic. The number of bands resulting in individual frequency estimates is not regular and is roughly five times larger towards the high frequency end. This choice is in part due to our effort to separate subtidal and intertidal bands which must be appreciably contaminated by the signals from strong oceanic tidal motions. (Electric fields of the motional type are generated by the displacement of oceanic water across the field lines of the Earth's main magnetic field.) The tidal bands as well as the relevant spectral estimates are indicated: clearly their energy density is substantially higher than in the adjacent intertidal bands. This excess is split between ionospheric signals from the daily variation and oceanic signals, the latter contributing mostly in **E_x** and **E_y**. Because of the mixing of these processes these bands are not used in the analysis that follows.

The reduced smoothness towards lower frequency is not simply due to the existence of the tidal peaks: in this area the the number of Fourier bands forming each estimate is reduced, being limited by the duration of the record available. But, the existence of another source of noise below 0.3 c.p.h. is strongly

Fig. 2 Sea floor electric and magnetic variations for two periods of one week: **H** horizontal magnetic field to the magnetic north, **D** to the east, **Z** downward; **E_x** horizontal electric field to the magnetic north, **E_y** to the east. Time and scales indicated.



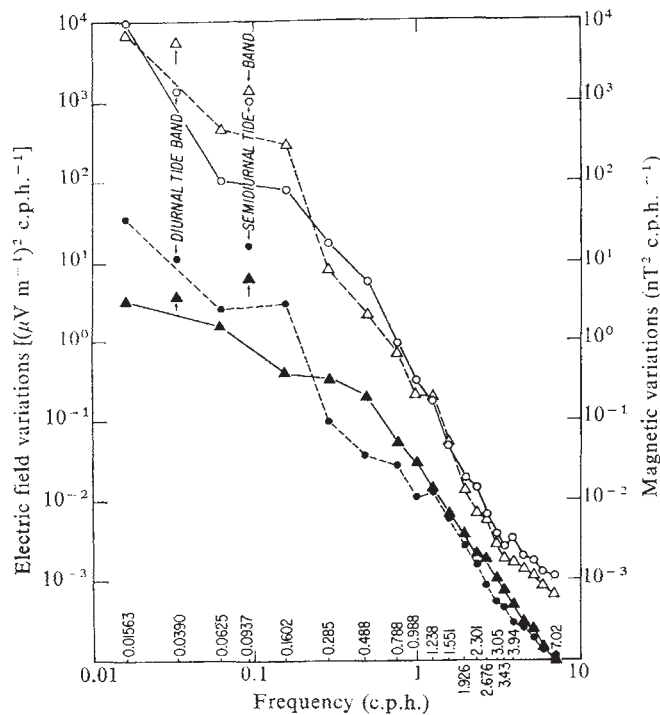


Fig. 3 Spectra of electric and magnetic variations. Notice special estimates corresponding to diurnal and semidiurnal tide bands, characterised by the higher energy density resulting from oceanic tidal motions. Δ , D ; $\circ$, H ; $\bullet$, E_x ; $\blacktriangle$, E_y .

suspected, with internal waves and internal tides as prime candidates.

The spectra of H , D and E_x , E_y show very similar behaviour, characterised by a very steep slope toward high frequencies. Also, features in the otherwise rather straight spectral curves, such as a jog around 1 c.p.h. track well, D with respect to E_x , and H with respect to E_y , as expected.

To the extreme right there is a sudden break toward a lesser slope in both H and D , occurring earlier in H than in D . This feature represents the instrumental noise contamination generated by the tape recorder stepping motor. Its greater effect on H compared to D simply relates to the closer proximity of suspension H to the recorder. (Shielding and spacing has suppressed this problem.)

In the following, the individual Fourier spectra are used to calculate cross-spectra and coherencies between all pairs of electric and magnetic components. From these estimates both impedances $Z(f)$ and admittances $A(f)$ are independently estimated according to

$$\begin{bmatrix} E_x \\ E_y \end{bmatrix} = \begin{bmatrix} Z_{xH} & Z_{xD} \\ Z_{yH} & Z_{yD} \end{bmatrix} \begin{bmatrix} H \\ D \end{bmatrix} \quad \text{or} \quad \mathbf{E}(f) = \mathbf{Z}(f) \mathbf{B}(f)$$

and

$$\begin{bmatrix} H \\ D \end{bmatrix} = \begin{bmatrix} A_{Hx} & A_{Hy} \\ A_{Dx} & A_{Dy} \end{bmatrix} \begin{bmatrix} E_x \\ E_y \end{bmatrix} \quad \text{or} \quad \mathbf{B}(f) = \mathbf{A}(f) \mathbf{E}(f)$$

where Z_{xH} etc., A_{Hx} etc., are the elements of the impedance and admittance tensors $\mathbf{Z}(f)$ and $\mathbf{A}(f)$, with f the frequency, $\mathbf{B}(f)$ the vector magnetic field (H , D) and $\mathbf{E}(f)$ the vector electric field (E_x , E_y).

If the assumption fundamental to the originally described MT method of an isotropic horizontally layered basement were to be satisfied, in the absence of ionospheric contamination¹⁴, (1) the diagonal elements of $\mathbf{Z}(f)$ and $\mathbf{A}(f)$ would be zero (the induced electric field is at right angle with the inducing field), (2) the absolute value of the antidiagonal elements would be equal and independent of the reference frame orientation and

(3) $\mathbf{Z}(f)$ would be the exact inverse of $\mathbf{A}(f)$.

In the presence of a contamination of electric and magnetic signals, equations (1) and (2) relate to the alternate assumptions that all noise affects only the electric, equation (1) or only the magnetic field, equation (2).

The hypothesis of isotropy can be easily tested visually by plotting the ratio of E to B at right angles to E as a function of the azimuth of E for various frequencies, see the result in Fig. 1. The moderate degree of elongation of the expected ideal circular patterns is consistent with a relatively minimal anisotropy. This situation greatly simplifies interpretation of the observed electromagnetic signals.

In the absence of precise knowledge concerning the distribution of noise in E and B we have assumed at first that both E and B are equally affected. We have in addition retained the combination of Z_{xD} and Z_{yH} which, according to Berdichevskiy¹⁵ minimises the effect of possible local heterogeneity of the conductivity distribution. The impedance thus arrived at and used in the interpretation is

$$Z'(f) = (Z_{eff}/A_{eff})^{\frac{1}{2}} \quad (3)$$

where Berdichevskiy's Z_{eff} effective¹⁵ is defined by

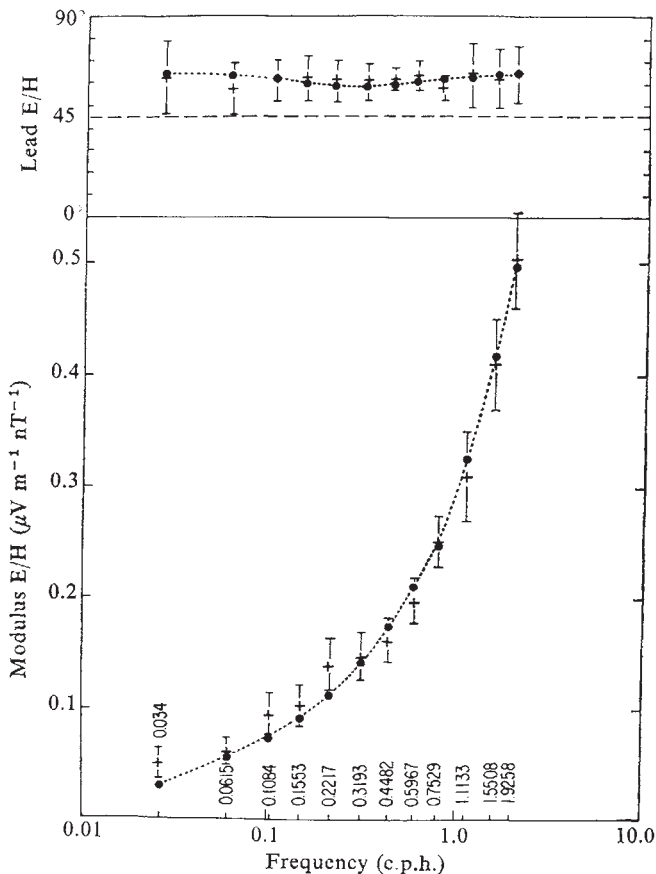
$$Z_{eff} = \frac{1}{2} (Z_{xD} - Z_{yH}) \quad (4)$$

and with

$$A_{eff} = \frac{1}{2} (A_{Hy} - A_{Dx}) \quad (5)$$

The resulting set of 12 impedances (sometimes called MT ratios, E/B) are plotted in amplitude and phase in Fig. 4 together with error bars at 95% confidence level (the frequency scale is logarithmic). The dotted line represents the impedance function characteristic of an isotropic horizontally layered model optimum with respect to the observed impedances.

Fig. 4 Impedances, or magnetotelluric ratios, derived from crossspectra between electric and magnetic signals, in amplitude (lower box) and phase (upper box). Error bars are indicated at the 95% confidence level. --- $\bullet$ ---, the optimum model; +, observations.



Conductivity distribution and depth

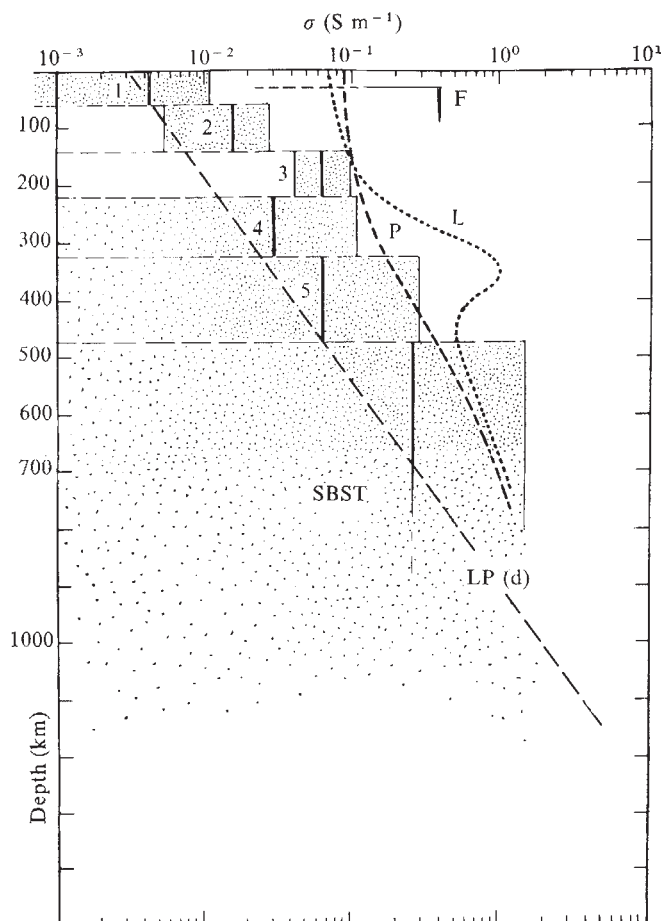
The method used to select the conductivity distribution with depth that best fits the observations will now be outlined briefly; 12 frequency estimates are available providing 12 amplitudes and 12 phases, thus a total of 24 constraints in a least square fit of the model predictions. The allowable number of model parameters thus must be substantially less than 24. With each horizontal layer involving thickness and conductivity—thus two parameters—a realistic model should include appreciably less than 12 layers. Using n layers with pre-determined thicknesses and conductivities the resulting impedances are calculated. The mismatch model to observation is estimated in terms of the variance between observed and predicted impedance functions.

The effect of changing the conductivity on the variance is then studied for each layer and improved conductivity values are updated accordingly. Convergence is rapid for the layers well characterised by the available cross-spectral estimates. From this rate of convergence, or equally well from the sensitivity of the variance to small conductivity changes, important implications result about the validity of the optimum model and the range of conductivity values acceptable in the various layers.

Following these guide lines we propose for the conductivity dependence with depth and for its range of uncertainty the five layer model with infinite half space substratum shown on Fig. 5. The layers are horizontal and isotropic. Their thicknesses are, from top downwards 60, 80, 80, 100 and 150 km respectively.

The layers best defined by the set of available impedances and

Fig. 5 Optimum six layer model of an isotropic, horizontally layered sea floor structure. Shaded area indicates range of uncertainty. Best resolved layer is layer 3, with a significantly high conductivity probably related to the asthenosphere. Estimates from other authors also shown.



in the order of decreasing resolution are 3, 2 and 4; the increased uncertainty in layer 1 adjacent to the ocean results from the high frequency cut off due to oceanic shielding, while decreasing resolution below layer 4 is a consequence of the low frequency impedance unreliability, itself related to the finite record length and increased oceanic motional noise. The influence of this signal is strongly supported by the much higher interpretability of the impedance estimates when noise contamination is assumed to be affecting mainly the electric field.

Earlier estimates of the Earth's electrical conductivity with depth are also shown in Fig. 5 as interpretative background, namely global averages proposed by Lahiri and Price¹⁶ (LP) and Parker¹⁷ (P), Larsen's profile below Hawaii¹⁸ (L) and Filloux's profile¹⁹ (F) 600 km offshore from Central California. The implied conductivity at the observation site is intermediate between that proposed by Lahiri and Price on the low side and that of Parker on the high side. A conductivity maximum is strongly suggested by the high conductivity average over an 80 km thickness in layer 3, with the value of 0.065 S m^{-1} . The actual conductivity maximum may, of course, be much higher, with a correspondingly smaller thickness. Its most probable depth of occurrence ranges within $180 \pm 40 \text{ km}$.

Although not very well resolved, the crustal conductivity is moderate to low, which is consistent with a sediment layer only a few hundred-metres thick and with the relatively old age of the local lithosphere: the matching seafloor spreading magnetic anomaly¹⁹ corresponds to reversal number 31 and to a plate age of 72 Myr (ref. 20).

The probability of a high conductivity tongue within layer 3 should be tested against processes implied by our present understanding of plate tectonics and ocean floor spreading, and against profiles of other physical properties of terrestrial materials over the same depth and, if possible, the same area.

A most attractive and immediate implication is that layer 3 contains the shear and partly molten layer or asthenosphere between mantle and lithosphere which permits plate motion. This view is in rough agreement with the range of occurrence of the oceanic low shear velocity layer²¹, namely 130–270 km. It is also compatible with Odegard's interpretation of the Cannikin/airborne experiment²² which suggests for an area broadly inclusive of our station, a low P-wave velocity layer extending from 210 to 320 km. Indirect estimates, however, of the lithospheric thickness^{23,24} suggest only 75 km for the local plate age. The discrepancy may be more apparent than real, relating to the still little known thickness of the asthenosphere in general and to the presently limited information concerning electrical conductivity of mantle materials, itself closely dependant on temperature and small changes in composition³.

The other MT profiles available from not-too-distant areas, that of Larsen (L), Fig. 5, and that of Filloux (F), like the present one predict or imply high conductivity layers and probable inversions. It is logical to attempt to relate these features and to tentatively predict that their significance may have something in common. Certainly the very high conductivity at a very shallow depth in the F profile is compatible with a much younger lithospheric age, about 32 Myr. The extremely high conductivity maximum in Larsen's model occurs at a considerably greater depth and may relate to the origin of the Hawaiian island chain.

In conclusion, and to give the foregoing analysis a realistic perspective, future MT sea floor work can be carried out with a considerably improved efficiency. Specifically, the following advantages can be readily attained: (1) longer records, up to one year, can be achieved with existing technology; (2) the frequency range can be readily extended upward by a factor of 4 and perhaps occasionally by a factor of 8 to 16; (3) broad coverage with instrumental arrays shall permit study of multidimensional structures such as spreading centres, subduction zones where old crust is consumed and trenches, island chains and arcs; and (4) magnetic activity, at a low during the present experiment shall progressively rise during the next five years, providing more energetic ionospheric fluctuations, thus

allowing improved resolution in general, and particularly in shallower crustal structures.

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¹ Tikhonov, A. N. *Doklady Akad. Nauk S.S.S.R.* 73, 275–295 (1950).

² Cagniard, L. *Annls Geophys.* 9, 95–125 (1953).

³ Shankland, T. J. S. *Phys. Earth planet Int.* 10, 209–219 (1975).

⁴ Cantwell, T. & Madden, T. R. *J. geophys. Res.* 65, 4202–4205 (1960).

⁵ Swift, C. M. thesis, M.I.T., (1967).

⁶ Hermance, J. F. *Trans. Am. Geophys. Union* 52, 1UGG (1971).

⁷ Vozoff, K. & Swift, C. M. *Intl. Upper Mantle Proj. Contrb.* 152 (1966).

⁸ Vozoff, K. *Geophysics* 37, 98–141 (1972).

⁹ Filloux, J. H. *Phys. Earth Planet Int.* 7, 323–338, (1973).

¹⁰ Filloux, J. H. thesis, Univ. California, La Jolla, California (1967).

¹¹ Cox, C. S., Filloux, J. H. & Larsen, J. C. *The Sea* 4, Part I, 637–693 (Wiley, New York, 1971).

¹² Bennett, D. J. & Filloux, J. H. *Rev. geophys. Space Phys.* 13, 197–239 (1975).

¹³ Filloux, J. H. *J. Geomag. Geoelectr.* 26, 269–279 (1974).

¹⁴ Swift, D. W. & Westcott, E. M. *J. geophys. Res.* 69, 4149–4154 (1964).

¹⁵ Berdichevskiy, M. N. & Demitriev, V. I. in *Geol Geoth. Str KAPG* (Adam, A. ed.) (Akademiai Kiado, Budapest, 1976).

¹⁶ Lahiri, B. N. & Price, A. T. *Phil. Trans. R. Soc. A237*, 509–540 (1939).

¹⁷ Parker, R. L. *Geophys. J.R. astr. Soc.* 22, 121–138 (1970).

¹⁸ Larsen, J. C. *Geophys. J.R. astr. Soc.* 43, 17–46 (1975).

¹⁹ Atwater, T. & Memard, H. W. *Earth planet. Sci. Lett.* 7, 445–450 (1970).

²⁰ Heirtzler, J. R., Dickson, G. O., Herron, E. M., Pitman III, W. C. & Le Pichon, X. *J. geophys. Res.* 73, 2119–2136 (1968).

²¹ Leeds, A., Knopoff, L. & Kausel, E. G. *Science* 186, 141–143 (1974).

²² Odegard, M. E. thesis, Univ. Hawaii (1975).

²³ Press, F. J. *geophys. Res.* 75, 6575–6581 (1970).

²⁴ Parker, R. L. & Oldenburg, D. W. *Nature* 242, 137–139 (1973).

Deep-sea carbonate and the deglaciation preservation spike in pteropods and foraminifera

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The aragonite compensation depth fluctuated considerably during the last 20,000 yr. This fluctuation culminated in a preservation spike at about 14,000 yr BP. The aragonite preservation stratigraphy parallels that in calcitic foraminifera, and constitutes part of a worldwide phenomenon signalling considerable changes in ocean chemistry and providing a useful tool for correlation.

THERE is abundant evidence that the effects of carbonate dissolution on the deep sea floor changed considerably as a result of Pleistocene climatic fluctuations. Within the last 20,000 yr, there has been a drastic worldwide drop of the aragonite compensation depth and of foraminiferal preservation levels during deglaciation, and a subsequent rapid rise to the present position. This fascinating phenomenon, which was a geologically almost instantaneous preservation pulse of 'spike', is the subject of this article.

The deglacial preservation spike was reported by the marine geology group at Kiel, working from the research vessel Meteor on the continental slopes off Portugal and Morocco^{1–4}. It expresses itself most commonly as a pteropod-rich layer, at the very end of the last glacial. The interpretation of this event is complicated by evidence for downslope redeposition. However, in many cases the pteropod layer does not seem to be associated with redeposited material, and a change in dissolution effects seems indicated as pointed out by Diester-Haass⁵. More recently, she proposed mass mortality of pteropods to explain the phenomenon⁶.

Recent evidence from the western equatorial Pacific^{7–8} indicates that the deglacial preservation spike is a worldwide event. Shackleton⁹ has drawn attention to this possibility and has commented on some geochemical implications of such an event.

Before turning to such implications, of which there are many, let us review briefly the evidence for the preservation stratigraphy from the last glacial to the present, that is, the last 20,000 yr.

Evidence for preservation stratigraphy

A drastic rise in the calcite compensation level at the glacial-Holocene boundary was recognised in the North Pacific¹⁰ on the basis of published foraminiferal data^{11,12}. Quite generally, there are fewer calcareous foraminifera in

Holocene sections of cores from the North Pacific than in the underlying glacial ones^{13,14}; the change has been dated at 12,500 yr BP¹⁵. Analogous observations were made elsewhere (East Pacific Rise¹⁶; Equatorial Pacific¹⁷; Andaman Sea¹⁸). The recognition of a Holocene dissolution pulse led to a reinterpretation of Arrhenius's carbonate cycles in the equatorial Pacific¹⁹ in terms of dissolution cycles^{20–23} with increased dissolution during interglacials, as suggested earlier by Olausson²⁴. Subsequently it was discovered that the dissolution cycles appear shifted with respect to the glacial-interglacial cycles^{25–27}. One type of shift can be produced by centering a carbonate preservation maximum on deglaciation, some 4,000 yr after the glacial maximum.

On examining the literature, it becomes evident that such a preservation spike exists not only in the eastern North Atlantic^{1–4} and in the west equatorial Pacific^{7,8}, but also in the Caribbean and in the Gulf of Mexico²⁸, on the western slope of India^{29,30}, and in many places in the deep sea, although here slow sedimentation rates and wide spacing of sampling intervals make recognition subject to doubt in each individual case (Atlantic: refs 31–33; Pacific refs 22, 23, 25).

The magnitude and the timing of the preservation pulse can be assessed. The pteropod data from the slope off NW Africa^{3,4} indicate that the euglacial ACD (lower depth limit of pteropods) stood at 1.5 km, the deglacial one at 3.5 km, and the postglacial one at 0.5 km (Fig. 1a). The corresponding ACD levels in the western equatorial Pacific are 1 km (?), 1.7 km, and 0.5 km (Fig. 1b). Similar fluctuations can be documented elsewhere and in shells other than pteropods (Table 1).

It will be noted that preservation during pre-deglacial

Table 1 Magnitude of preservation spike in calcareous shells

Type of level	Area	Depth (km) during			Source
		Post-G.	De-G.	Eu-G.	
ACD	Off NW Africa	0.5	3.5	1.5	Refs 3, 4
ACD	Off Portugal	1.1	3.6	2.8	Ref. 2
ACD	Off W India	0.3	2.5	1.2	Ref. 29
ACD	W. Eq. Pacific	0.5	1.7	1(?)	This article
RCD	W. Eq. Pacific	2.8	3.8	3.1	This article

ACD: lower depth limit of pteropods. RCD: lower depth limit of *Globigerina rubescens*, a planktonic foraminifer that is highly susceptible to dissolution. Post-G.: postglacial.

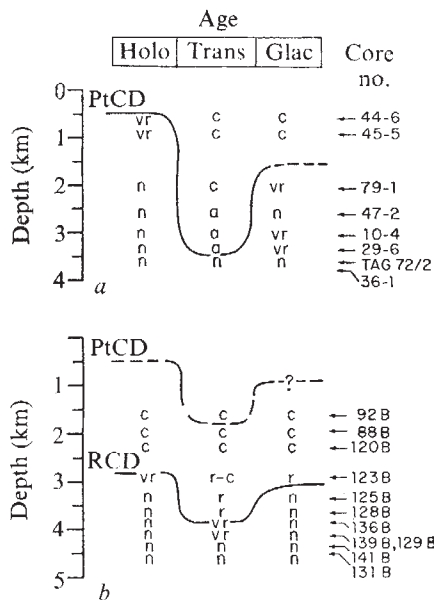


Fig. 1 Excursion of the compensation depth for pteropods (PtCD) and for *Globigerina rubescens* (RCD) during the transition period from glacial to Holocene time. a, Off NW Africa, based on data given by Diester-Haass (refs 3 and 4). b, Western equatorial Pacific, own data.

(‘euglacial’) is indeed better than during postglacial time, in agreement with earlier assessments based on accumulation rate comparisons²⁰. However, the preservation story is clearly more complicated than a simple glacial–interglacial dichotomy would suggest.

The timing of the preservation spike is of crucial importance if the geochemical driving mechanisms are to be discovered. The data in Kudrass² suggest that the spike is centred in 13,500 yr BP. Our own data from the equatorial Pacific⁸ and those of Chen and of van Donk (in ref. 20) indicate maximum preservation during maximum change in the oxygen isotope signal. In the Gulf of Mexico, this period of maximum change is dated at about 15,000 yr to 12,500 yr BP^{34,35}, although the exact timing is in some dispute. In the west equatorial Pacific, the period of maximum change (and the preservation spike) seems to be close to 14,000 yr BP (ref. 8). The same period is centred between 13,500 and 14,000 yr BP in the dated isotope sequence of a core in the equatorial Atlantic³⁶. One may conclude that the spike has its maximum at about 14,000 yr BP.

The spike is narrow, perhaps no more than 1,000 yr wide. Considering the effects of bioturbation, the resolution of such an event in deep sea cores represents the limit for this type of record. A further constraint on the timing is given by the rapid increase of dissolution following this spike, starting at about 13,000 yr BP^{14,15}. In fact, the very recording of the rapid onset of the dissolution pulse in deep sea sediment, despite benthic mixing and the presence of good preservation immediately preceding it, strongly suggests a flip-flop situation, with a dissolution spike immediately following a preservation spike.

Geochemical and stratigraphical implications

What are some of the geochemical and stratigraphic implications of the deglacial preservation spike?

Fundamentally, we can enhance preservation of carbonate in deep waters by increasing saturation, by decreasing the speed of bottom currents, or by suddenly increasing sedimentation rate, for example, by redeposition. I suggest that a combination of these factors is at work, rather than any one process. First, let us examine saturation.

The thermodynamic equations on which saturation determination depends contain the following alterable terms³⁷: (1) partial pressure of CO₂, and (2) temperature and chlorinity. Availability of CaCO₃ (3) enabling reactions to proceed towards equilibrium, also is a variable in the real ocean. We see that the first item refers to a property of the atmosphere, the second to the water itself, and the third to conditions at the sea floor.

To enhance preservation then, the following possibilities arise:

- Lowering of the CO₂ pressure in the atmosphere. Shackleton⁹ has suggested that the rapid growth of the biosphere during deglaciation would lead to extraction of CO₂ from the atmosphere–ocean system, and hence result in improved preservation of carbonate. Both a change from glacial aridity to postglacial humidity in tropical latitudes³⁸ and the recession of tundra and ice would be responsible for this ‘biosphere effect’. If one adds the dead carbon (‘thanatosphere’) that would build up together with the growing forests and below the rising sea level, this effect could indeed be substantial—of the order of 1% of the entire ocean–atmosphere CO₂ content. In areas of gentle depth gradients of saturation, such a change could result in marked excursions of the dissolution level. One problem with accepting Shackleton’s biosphere effect as the main or sole factor is the timing of the major changes in floral cover. Apparently this major change is not centred on 14,000 yr BP, but rather occurs between 13,000 yr and 10,000 yr ago^{39,40}, that is, during the dissolution pulse rather than the preservation spike.

- Increase in temperature of deep ocean waters. Weyl⁴¹ speculated that abyssal waters would have warmed during glacials and Worthington⁴² pointed out that a low-salinity meltwater lid due to deglaciation would lead to stagnant bottom waters, affecting carbonate preservation. There is indeed evidence for such a glacial warming of deep water^{43–45}, at least in the Atlantic Ocean. This ‘glacial bottom water effect’ does not seem to be focused enough to account for the preservation spike, although it must be taken into account in the overall glacial–postglacial preservation fluctuations.

- Decrease of the chlorinity of deep ocean waters. The addition of meltwater eventually decreases chlorinity by some 3%. This is most likely done with run-off water, which brings its own dissolved load of calcium carbonate. The exact effect on saturation of the ocean as a whole would seem to depend on the magnitude of this load, which would depend on the rate of recycling of the water, through continuous evaporation and precipitation, during the time of net addition to the ocean, and on the rate of concomitant chemical erosion.

- Increase of availability of CaCO₃ in abyssal depths. There is considerable evidence for large-scale redeposition of shallow water sediments in the Late Pleistocene, including pteropod-rich sediments^{2–4,15,29,38}. Such transport makes carbonate particles available at abyssal depths. Here bottom waters are greatly undersaturated and are able to dissolve such particles at a maximum rate. On rising, therefore, these waters will quickly reach saturation values which preclude rapid attack of calcareous shells. The timing of this ‘neutralisation’ of abyssal waters seems to be correct.

Two more possibilities can be added, considering that the ocean is not in equilibrium:

- Decrease in the rate of extraction of carbonate in surface waters, by calcareous organisms. A reduction of precipitation of shells in surface waters will increase the overall saturation of the ocean with the respective substance and therefore enhance preservation^{46,47}. (Thus, the relationship between overall fertility and preservation of biogenous sediments is exactly the reverse of that usually suggested.) It is possible that a rapid dumping of meltwater, combined with incomplete mixing, would temporarily reduce produc-

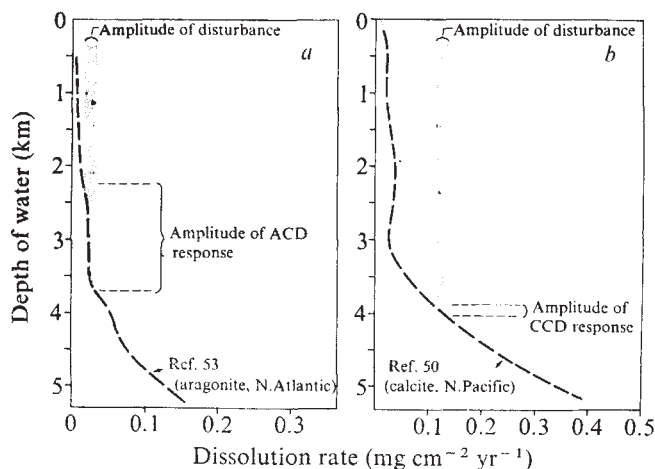


Fig. 2 Compensation depth fluctuations as a response to a disturbance (for example change in supply). The amplitude of the response is strongly governed by the depth gradient of dissolution, and its relationship to the disturbance. The reaction of the system to a given disturbance provides insights into the state of undersaturation.

tivity, although there is no evidence for such a 'fertility effect'.

● Increase in the rate of supply of alkalinity to the ocean as a whole. The redeposition of shelf carbonates (the fourth item, above) constitutes addition of alkalinity. Also, the alkalinity supply may be increased during deglaciation by an increase in the suspended and dissolved load of river input, through erosion of glacial weathering products (loess, calcareous desert crusts, morainal debris). If conditions fluctuated between arid and humid and before the plant cover could re-establish itself, this would provide for maximum erosion. Also, sea level was still considerably depressed, leaving the upper shelf areas exposed for erosion, and facilitating bypassing of the lower ones by the sediment introduced. Considering the evidence for transport of terrigenous and shelf deposits into abyssal waters, the 'neutralisation effect' could have operated earlier, on a reduced level, throughout glacial conditions, aided by additional supply whenever arid conditions changed to humid.

It should be noted that the type of stagnation visualised by Worthington⁴², due to a hypothetical meltwater lid, should lead to a decrease in bottom currents (enhancing preservation) but also to a build-up of CO₂ (enhancing dissolution). In this scenario, when ocean mixing finally catches up with the meltwater, CO₂ is released to the atmosphere, and a climatic optimum ensues. Increased CO₂, together with rapid extraction of carbonate on the flooded shelves, probably accounts for the dissolution pulse following the preservation spike.

It is obviously tempting to go on speculating about the relationship between CO₂ fluctuations and preservation and dissolution pulses, especially in view of the man-made CO₂ input to the atmosphere and its presumed ultimate effects on carbonate deposition and climate⁶. Suffice it to say that the type of analysis presented here, which is an example of preservational stratigraphy, has considerable potential for palaeoclimatic studies in the deep sea as well as in shallow waters⁴³.

The most obvious use of the preservation spike is for correlation and dating of deep sea cores. Relative preservation aspects can be determined extremely rapidly by visual inspection, for example, by noting the degree of fragmentation of foraminifera in the fine sand fraction. Thus, the peak preservation is easily found. We have already made considerable use of this method in the deep-sea sediment

laboratory at the Scripps Institution of Oceanography. A word of caution is in order, however. Before determining sedimentation rates by this method, the displacement of a spike by benthic mixing must be considered. This displacement is downward, by up to one mixed layer thickness, depending on the ratio between the thickness of the layer and the thickness corresponding to the actual duration of the event. For example, a rate of 1.93 cm per 1,000 yr would be calculated for the pteropod-containing core Bx 92 (Fig. 1b), if the centre of the pteropod preservation spike at 27 cm is divided by 14,000 yr, the estimated age of the event. Allowing for a mixed layer of 6 cm (ref. 17), and a duration of the spike of 2,000 yr (3–4 cm), the offset is 4.5–4 cm (mixed layer thickness minus one-half the width of the event, see ref. 49). Thus, the actual sedimentation rate, under these assumptions, is closer to 1.6 cm per 1,000 yr than to 1.9 cm per 1,000 yr, a non-trivial correction. The problem is not restricted to the dating method presented here, but is of a general nature. It is negligible only in cores with high sedimentation rates.

One final note of caution in interpreting the significance of preservation fluctuations: a change in preservation is a result of both the condition of the system (sensitivity, that is, depth gradient of potential dissolution rate) and the strength of the agent of change (disturbance, resulting in a change in the record). Depth gradients of dissolution can change both in space^{50–53} and in time^{54–56}. Disturbances of similar magnitude, therefore, can produce greatly differing amplitudes in preservational signals (Figs 2a, b).

In fact, the response amplitudes of high frequency ACD and CCD fluctuations through time may be a useful tool for determining variations in the strength of the depth gradient of undersaturation in the deep ocean. For example, relatively small disturbances in Late Cretaceous carbonate sedimentation in the central Atlantic apparently were responsible for large CCD excursions⁵⁷ presumably due to high sensitivity of the system (gentle dissolution depth gradient). In comparing the record of marginal seas to that of the open ocean, it must be kept in mind that these seas may not play exactly the same tune as the open ocean. Thus the Late Pleistocene and Holocene preservation stratigraphies of the Mediterranean, the Red Sea, the Sea of Japan and the Arctic Ocean are entirely different from the one described here (see refs 28, 58, 59).

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- 1 Thiede, J. *Meteor. Forsch. Ergebn. Reihe C* 7, 15–102, (1971).
- 2 Kudrass, H.-R. *Meteor. Forsch. Ergebn. Reihe C* 13, 1–63 (1973).
- 3 Diester-Haass, L., Schrader, H.-J. & Thiede, J. *Meteor. Forsch. Ergebn. Reihe C* 16, 19–66 (1973).
- 4 Diester-Haass, L. *Meteor. Forsch. Ergebn. Reihe C* 20, 1–32 (1975).
- 5 Diester-Haass, L. & van der Spoel, S. *Palaeogeogr. Palaeoclimat. Palaeoecol.* (in the press).
- 6 *Fate of Fossil Fuel CO₂ in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
- 7 Berger, W. H. in *Fate of Fossil Fuel CO₂ in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
- 8 Berger, W. H. & Killingley, J. S. *Science* 197, 563–566 (1977).
- 9 Shackleton, N. J. in *Fate of Fossil Fuel CO₂ in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
- 10 Berger, W. H. *Mar. Geol.* 8, 111–138 (1970).
- 11 Nayudu, Y. R. *Science* 146, 515–517 (1964).
- 12 Saidova, H. M. *Progr. Oceanography* 4, 143–152 (1965).
- 13 Bandy, O. L. *Progr. Oceanography* 4, 27–49 (1967).
- 14 Barnard, W. D. & McManus, D. A. *Geol. Soc. Am. Bull.* 84, 2097–2100 (1973).
- 15 Duncan, J. R., Fowler, G. A. & Kulm, L. D. *Geol. Soc. Am. Bull.* 81, 561–566 (1970).
- 16 Broecker, W. S. & Broecker, S. *Soc. Econ. Geol. Paleont. Spec. Publ.* 20, 44–57 (1974).
- 17 Berger, W. H. in *Benthic Processes and Geochemistry of Interstitial Waters of Marine Sediments* (ed. Fanning, K. A. & Manheim, F. T.) (Joint Oceanogr. Congress, Edinburgh, in the press).
- 18 Frerichs, W. E. *Science* 159, 1456–1458 (1968).
- 19 Arrhenius, G. *Rep. Swed. Deep Sea Exped.* 5, 1–228 (1952).
- 20 Broecker, W. S. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 239–265 (Yale University, 1971).
- 21 Berger, W. H. *J. Foraminif. Res.* 3, 187 (1973).
- 22 Thompson, P. R. & Saito, T. *Geology* 2, 333–335 (1974).

- 23 Thompson, P. R. *J. Foram. Res.* 6, 208–227 (1976).
- 24 Olsson, E. *Progr. Oceanography* 3, 221–252 (1965).
- 25 Luz, B. & Shackleton, N. J. *Cushman Found. Foram. Res. Spec. Publ.* 13, 142–150 (1975).
- 26 Piasias, N. G., Heath, G. R. & Moore, T. C. *Nature* 256, 716–717 (1975).
- 27 Moore, T. C., Piasias, N. G. & Heath, G. R. in *Fate of Fossil Fuel CO₂ in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
- 28 Chen, C. *Nature* 21, 1145–1149 (1968).
- 29 von Stackelberg, U. *Meteor. Forsch. Ergebn. Reihe C* 9, 1–73 (1972).
- 30 Zobel, B. *Meteor. Forsch. Ergebn. Reihe C* 12, 9–73 (1973).
- 31 Ruddiman, W. F. *Geol. Soc. Am. Bull.* 82, 282–302 (1971).
- 32 Thiede, J. *1973 Geol. Soc. Am. Bull.* 84, 2749–2754 (1973).
- 33 Damuth, J. E. *Deep-Sea Res.* 22, 725–743 (1975).
- 34 Kennett, J. P. & Shackleton, N. J. *Science* 188, 147–150 (1975).
- 35 Emiliani, C., Gartner, S., Lidz, B., Eldridge, K., Elvey, D. E., Huang, T. C., Stipp, J. J. & Swanson, M. F. *Science* 189, 1083–1088 (1975).
- 36 Wiseman, J. D. H. *Rep. Swed. Deep Sea Exped.* 288–354 (1965).
- 37 Gieskes, J. M. in *The Sea* (ed. Goldberg, E. D.) 5, 123–151 (1974).
- 38 Damuth, J. E. & Fairbridge, R. W. *Geol. Soc. Am. Bull.* 81, 189–206 (1970).
- 39 van der Hammen, T., Wijnstra, T. A. & Zagwijn, W. H. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 391–424 (Yale University, 1971).
- 40 Wright, H. E. in *The Late Cenozoic Glacial Ages* (ed. Turekian, K. K.) 425–464 (Yale University, 1971).
- 41 Weyl, P. K. *Meteorol. Monogr.* 9, 37–62 (1968).
- 42 Worthington, L. V. *Meteorol. Monogr.* 8, 63–67 (1968).
- 43 Duplessy, J. C., Chenouard, L. & Vila, F. *Science* 188, 1208–1209 (1975).
- 44 Streeter, S. S. *Quat. Res.* 3, 131–141 (1973).
- 45 Schnitker, D. *Nature* 248, 385–387 (1974).
- 46 Berger, W. H. *Geol. Soc. Am. Bull.* 81, 1385–1402 (1970).
- 47 Berger, W. H. & Roth, P. H. *Rev. Geophys. Space Phys.* 13, 561–585, 624–635 (1975).
- 48 Alexandersson, E. T. *Science* 189, 47–48 (1975).
- 49 Berger, W. H. & Heath, G. R. *J. Mar. Res.* 26, 134–143 (1968).
- 50 Peterson, M. N. A. *Science* 154, 1542–1544 (1966).
- 51 Ben-Yaakov, S., Ruth, E. & Kaplan, I. R. *Science* 184, 982–984 (1974).
- 52 Ben-Yaakov, S., Ruth, E. & Kaplan, I. R. *Deep Sea Res.* 21, 229–243 (1974).
- 53 Milliman, J. D. *Geology* 3, 461–462 (1975).
- 54 Berger, W. H. *Nature* 236, 392–395 (1972).
- 55 van Andel, T. J. H., Heath, G. R. & Moore, T. C. *Geol. Soc. Am. Memoir* 143, 1–134 (1975).
- 56 Heath, G. R., Moore, T. C. & van Andel, T. J. H. in *Fate of Fossil Fuel CO₂ in the Ocean* (eds Anderson, N. R. & Malahoff, A.) (Plenum, New York, 1977).
- 57 Berger, W. H. & von Rad, U. *Init. Rep. Deep Sea Drilling Project* 14, 787–954 (1972).
- 58 Ujiié, H. & Ichikura, M. *Trans. Proc. Paleont. Soc. Japan, N. S.* 91, 137–150 (1973).
- 59 Herman, Y. *Marine Geology and Oceanography of the Arctic Seas* 283 (Springer, Heidelberg, 1974).

Structure of a dinucleoside phosphate–drug complex as model for nucleic acid–drug interaction

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The crystal structure of a 3 : 2 complex of the frameshift mutagen proflavine with the dinucleoside phosphate cytidyl-3'-5'-guanosine has been determined. The complex has one drug molecule intercalated between Watson–Crick base pairs of the nucleotide duplex. The other two proflavine molecules are bound to the exterior of the miniature double helix. The orientation of the base pairs in this miniature double helix has aspects similar to that found in RNA II.

It is well established that many drugs and carcinogens act by binding to nucleic acids; both nuclear double-stranded DNA and RNA have been shown to be targets for such interactions^{1,2}. Many such drugs are characterised by a planar aromatic structure, and the acridines, in particular, have been the subject of much study¹. The concept of drug intercalation between adjacent base pairs of the nucleic acid, first introduced by Lerman³, accounts satisfactorily for many observed properties of drug–nucleic acid complexes, and is widely accepted. Intercalation is believed to be responsible for the activity of many of these drugs as frameshift mutagens^{4–6}. In addition, intercalation, and the subsequent unwinding of nucleic acids, has emerged as an important tool in the study of superhelicity^{7,8}.

Intercalation necessitates an alteration in the geometry of the double helix at (and, presumably, adjacent to) the actual binding site. In normal B DNA the turn angle of rotation between adjacent nucleotide residues is 36°. In the original model for intercalation³ the helix uncoils by 45° from this value. Subsequent studies^{9–12} using various drugs including proflavine provide support for the intercalation hypothesis although the degree of concomitant unwinding remains uncertain with values ranging from –45° to 12°. Linked atom refinement techniques¹³ have been used to define further both the degree of unwinding as well as the exact conformational features of DNA complexed with proflavine.

Proflavine (Fig. 1) is involved in more than one mode of

binding^{1,14}. In addition to the strong intercalative process which occurs at low drug levels, there is a weaker binding of the drug, presumably external to the helix. Temperature-jump relaxation kinetics data^{15,16} show, however, that the strong binding process is itself a complex one, involving as an initial step, externally-bound drug molecules. It is apparent that a realistic visualisation of proflavine–DNA binding should involve both factors.

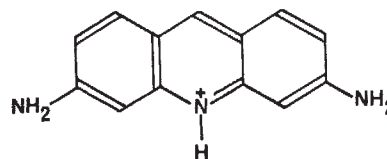


Fig. 1 A proflavine cation.

We have determined the crystal structure of a complex between proflavine and cytidyl-3'-5'-guanosine (CpG) (Fig. 2), in the hope that this would serve as a model for proflavine–nucleic acid interactions. We were encouraged in this belief by analyses of two other dinucleoside monophosphates^{17,18}, which showed the features of an RNA-like miniature double helix, and of several drug–dinucleoside phosphate complexes^{19,20} which demonstrated intercalative binding. The proflavine–CpG complex was deliberately produced without any heavy atoms so as to avoid the possibility that they might perturb the structure.

Crystallographic analysis

Large, well formed single crystals of the complex were produced from an aqueous solution of proflavine hemisulphate and free acid CpG. The crystals showed triclinic symmetry, space group *P*1, with cell dimensions *a* = 16.098 Å, *b* = 16.755 Å, *c* = 12.933 Å, α = 112.67°, β = 113.69° and γ = 99.36°. As has been observed with other dinucleoside phosphates²¹, however, the diffraction pattern displays pronounced pseudosymmetry such that it

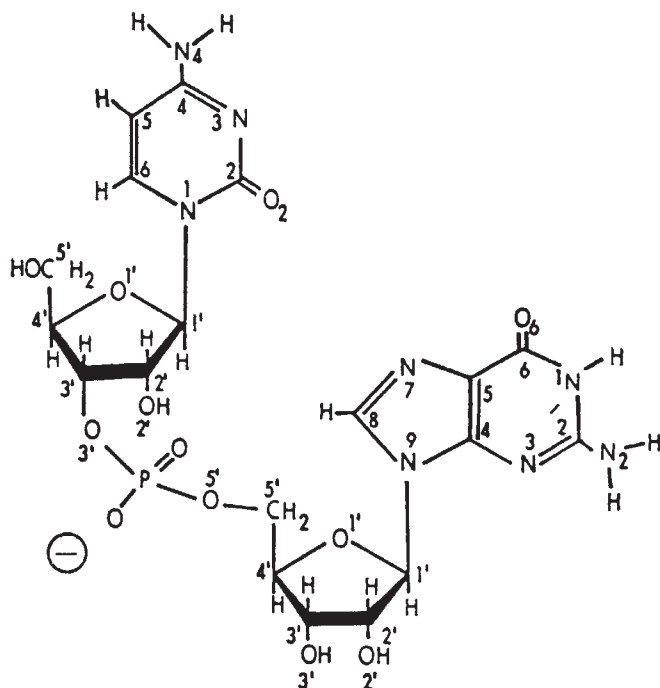


Fig. 2 The cytidyl 3',5'-guanosine anion.

was possible to index the reflections in a monoclinic cell, space group $C2$, with cell dimensions $a = 29.487$ Å, $b = 12.933$ Å, $c = 16.883$ Å and $\beta = 121.94^\circ$. Because of this ambiguity, the intensity data were measured on the basis of the space group $P1$. A crystal was sealed in a glass capillary in which mother liquor was included.

A total of 5,621 independent reflections was measured, to a limit of 1 Å resolution, on an automatic four-circle diffractometer using $\text{CuK}\alpha$ radiation. Of these, 4,073 reflections were considered to have intensity significantly greater than background. In addition, Friedel mates for 356 reflections were measured and the agreement, R_{sym} , for these 356 Friedel pairs was found to be 0.038. When the reflection data were transformed to space group $C2$, the agreement among the symmetry-related reflections was 0.139. The fact that the agreement for symmetry related reflections for space group $P1$ was significantly better than for space group $C2$ was taken as a strong indication that space group $P1$ is more probable. Accordingly, the structural analysis was carried out in the latter space group.

Numerous attempts at structure solution by direct and search methods were unsuccessful. The structure was eventually solved by location of the phosphorous atoms using the high resolution Patterson technique²², followed by a number of cycles of (E and F) Fourier syntheses to determine the rest of the structure. Early stages of the analysis of the crystal structure were greatly facilitated by the use of the interactive computer graphics system²³ at the Institute for Cancer Research. This permitted the rapid detailed evaluation of possible structural fragments at each stage of the analysis. Least squares refinements, as yet incomplete, have reached an R of 0.155 and the overall structure is well ordered; only a few atoms display high thermal motion.

Proflavine interacts with miniature double helix in several ways

A projection of the structure is illustrated in Fig. 3. It is a 3:2 proflavine-CpG complex and represents the first reported crystal structure of drug-dinucleotide complex which does not have 2:2 stoichiometry. The two dinucleotides are hydrogen bonded to form a miniature double

helix with Watson-Crick base pairs, separated by 6.8 Å. One of the proflavine molecules is symmetrically intercalated between the base pairs and protrudes into the wide groove. Each amino group of this proflavine forms a hydrogen bond to a phosphate oxygen on either side of the duplex. These interactions, therefore, probably serve to stabilise the observed symmetry of intercalation. The other two proflavine molecules stack asymmetrically above and below the miniature double helix. The asymmetry is such that these proflavines tend to stack more over the guanine residues thereby preserving the pseudo-twofold symmetry which may be described as an axis perpendicular to the helix axis and lying along the C(9)-N(10) vector of the intercalated proflavine molecule.

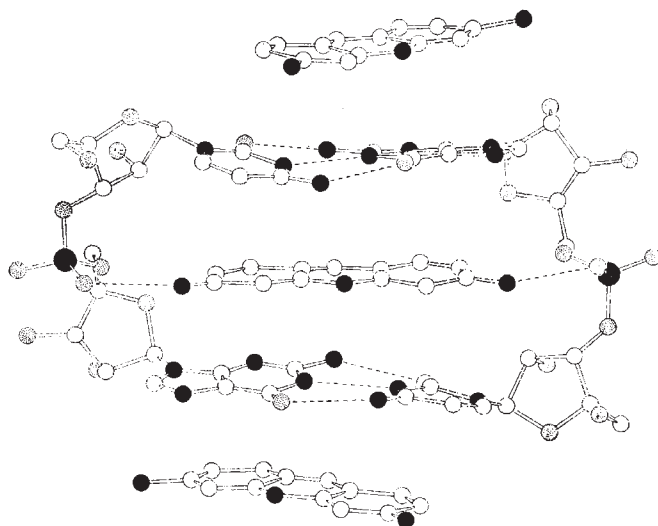
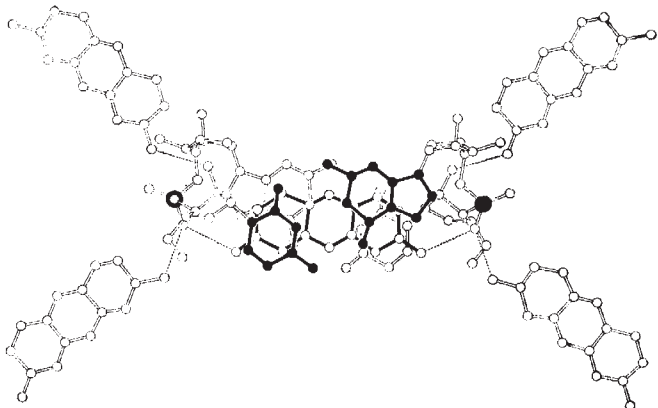


Fig. 3 A view of the structure of the complex, tilted slightly from the view parallel to the mean planes of the base pairs. Dotted lines represent hydrogen bonds. Nitrogen and phosphorus atoms are black and oxygen atoms are stippled.

Inspection of the non-intercalated proflavine molecules (translated to adjacent unit cells) shows that the molecules also bind externally to the duplex through a system of hydrogen bonding (Fig. 4). The external binding to the backbone of the duplex may be described by four unique hydrogen bonds—two from proflavine amino groups to phosphate oxygens and two more from proflavine amino groups to the O(2') hydroxyl oxygens of the cytosine riboses. Note that the phosphate oxygens that are hydrogen bonded in this manner are also those that are hydrogen bonded to the amino groups of the intercalated proflavine

Fig. 4 A view of the structure, looking perpendicular to the planes of the base pairs. The two phosphorus atoms are shown in black, one as a heavy open circle and the other as a large solid circle. The atoms of the uppermost base pair are also in black and the intercalated proflavine has black bonds.



molecule. The packing in the crystal structure is such that the non-intercalated proflavines link adjacent base-paired duplexes by these external hydrogen bonds; when one amino group of an external proflavine molecule is hydrogen bonded to a phosphate oxygen, the other amino group of that proflavine molecule is hydrogen bonded to an O(2)' hydroxyl oxygen of a neighbouring duplex (Fig. 5). The overall effect is a pattern of extensive cross-linking between adjacent miniature double helices.

The crystal structure of the complex is heavily hydrated, with 24 water molecules associated with each unit. This large degree of hydration lends credence to the relevance of this structure to nucleic acid-drug complexes. A full description of the many interactions involved will be published elsewhere.

Proflavine stacks with base pairs and also with other proflavines

THE sequence of stacked polycyclics is proflavine-base pair-intercalated proflavine-base pair-proflavine-base pair-intercalated proflavine, and so on, so that proflavine-proflavine stacking also occurs (Fig. 5). In this case the long axes of the proflavine molecules lie at approximately 83° to each other. This is a different situation from that found in proflavine hydrochloride²⁴ where this angle is 180° and in proflavine hemisulphate where this angle is 150° (refs 2, 25). The skewness of stacking of proflavine molecules probably results from the fact that the terminal amino groups of proflavine are hydrogen bonded to phosphate oxygen atoms and to ribose O(2)' hydroxyl groups. These hydrogen bonds position the molecule with respect to the base pairs and, to satisfy the crystallisation conditions, proflavine-proflavine stacking occurs as best it may.

The 3:2 nature of this complex, containing two mononucleotides of CpG (Fig. 2), suggests that one of the three proflavine molecules carries no charge. By symmetry one would expect this to be the intercalated proflavine molecule. Assignment of these charges must await further refinement and the location of hydrogen atoms.

The miniature double helix has unexpected features

Each base pair is twisted in a propeller-like fashion, much as has been found in other dinucleoside phosphate structures. The spacing between base pairs is 6.8 Å, as would be expected for a complex of this sort. What is not expected, however, is that the orientation of the two base pairs has aspects similar to that found in normal, uncomplexed RNA 11 (ref. 26). That is, the angle between the vectors connecting the glycosidic carbon atoms within each base pair is 33° , not the 8° – 10° values found in the structures of iodo-CpG-ethidium bromide and iodo-CpG-9-amino-acridine.

Moreover, instead of having C(3)' *endo* 3'-5' C(2)' *endo* mixed sugar pucker²⁷, all the sugars are C(3)' *endo*. The stretching (rather than unwinding) of this miniature helix is accomplished by systematic changes in the backbone and glycosidic angles. The implications of this surprising result with respect to the unwinding of polynucleotide structure are under investigation.

The results of this analysis allow us to explain various phenomena associated with proflavine complexation to nucleic acids. We have defined in geometric terms two modes of binding: (1) symmetrical intercalation with hydrogen bonding to the phosphate groups, and (2), hydrogen bonding to phosphate and ribose O(2)' hydroxyl atom external to the helix. It is known that when acridines are used to stain cells, the RNA complex shows a red fluorescence whereas the DNA has a green fluorescence¹.

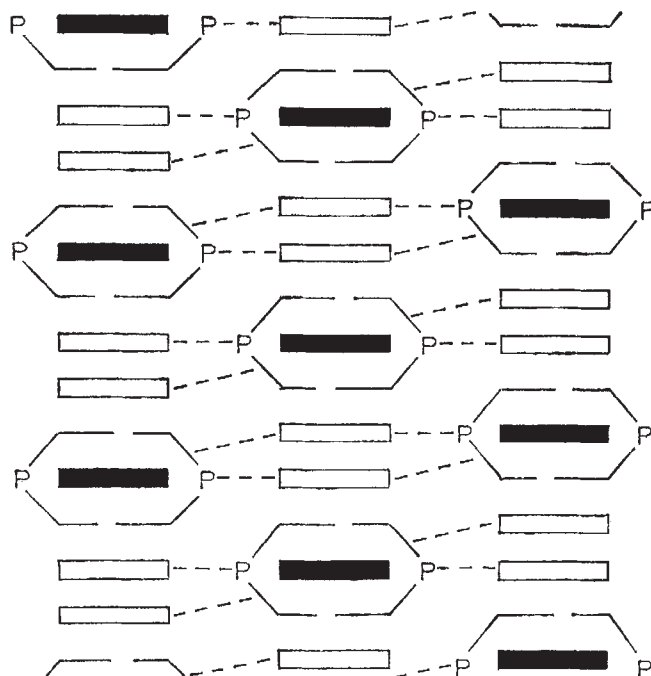


Fig. 5 A diagrammatic representation of the packing of the CpG and proflavine ions in the crystal lattice.

This difference may be explained, in part, by the hydrogen bonding between the O(2)' hydroxyl of the ribose and the amino nitrogen of the proflavine since there is no O(2)' hydroxyl group in DNA. The structure also shows the nature of the aggregation of the proflavine molecules themselves (see Fig. 5). Finally, the similarity of the orientation of the overlapping base pair planes to those in RNA 11 (as measured by the vectors between the C(1)' glycosidic atoms), implies that proflavine does not unwind the helix.

This work was supported by grants CA-05322, CA-10925, GM-21589, CA-06927 and RR-05539 from the US NIH, an appropriation from the Commonwealth of Pennsylvania, and by grants from the Cancer Research Campaign, the SRC, and the Lawson Tait Trust. S.N. is grateful to the Royal Society and the Cancer Research Campaign for travel grants, and to the Institute for Cancer Research for their hospitality. We also thank G. M. Sheldrick, M. Spencer, L. Wakelin and M. Waring for helpful comments. *Note added in proof:* We have been informed by Alden and Arnott that they have developed a model for A DNA-proflavine binding which has many features similar to our structure. Sobell *et al.* have given a preliminary structural report of a 2:2 proflavine-iodo CpG complex (American Crystallographic Association meeting, August 1977).

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- 1 Peacocke, A. R. in *The Acridines* (ed. Acheson, R. M.) (Wiley, New York, 1973).
- 2 Neidle, S. & Jones, T. A. *Nature* **253**, 284–285 (1975).
- 3 Lerman, L. S. *J. molec. Biol.* **3**, 18–30 (1961).
- 4 Drake, J. W. & Baltz, R. H. *A. Rev. Biochem.* **20**, 11–37 (1976).
- 5 Roth, J. R. *A. Rev. Genet.* **8**, 319–346 (1974).
- 6 Crick, F. H. C., Barnett, L., Brenner, S. & Watts-Tobin, R. J. *Nature* **192**, 1227–1232 (1961).
- 7 Wang, J. C. *J. molec. Biol.* **89**, 783–801 (1974).
- 8 Waring, M. J. *J. molec. Biol.* **54**, 247–279 (1970).
- 9 Fuller, W. & Waring, M. J. *Ber. Bunsenges. Physik Chem.* **68**, 805–808 (1964).
- 10 Lerman, L. S. *J. cell. comp. Physiol.* **64**, Suppl. 1, 1–18 (1964).
- 11 Neville, D. M. & Davies, D. R. *J. molec. Biol.* **17**, 57–74 (1966).
- 12 Bond, P. J., Langridge, R., Jennett, K. W. & Lippard, S. J. *Proc. natn. Acad. Sci. U.S.A.* **72**, 4825–4829 (1975).
- 13 Alden, C. & Arnott, S. *Nucleic Acid Res.* **2**, 1701–1717 (1975).
- 14 Peacocke, A. R. & Skerrett, N. J. H. *Trans. Faraday Soc.* **52**, 261–279 (1956).
- 15 Li, H. J. & Crothers, D. M. *J. molec. Biol.* **39**, 461–477 (1969).
- 16 Ramstein, J. & Leng, M. *Biophys. Chem.* **3**, 234–240 (1975).
- 17 Seeman, N. C., Rosenberg, J. M., Suddath, F. L., Kim, J. J. P. & Rich, A. *J. molec. Biol.* **104**, 109–144 (1976).

- ¹⁸ Rosenberg, J. M., Seeman, N. C., Day, R. O. & Rich, A. J. *molec. Biol.* **104**, 145–167 (1976).
¹⁹ Tsai, C. C., Jain, S. C. & Sobell, H. M. *Proc. natn. Acad. Sci. U.S.A.* **72**, 628–632 (1975).
²⁰ Sakore, T. D., Jain, S. C., Tsai, C. C. & Sobell, H. M. *Proc. natn. Acad. Sci. U.S.A.* **74**, 188–192 (1977).
²¹ Hingerty, B. *et al.* *Biopolymers* **14**, 227–236 (1975).
²² Sussman, J. L., Seeman, N. C., Kim, S. H. & Berman, H. M. *J. molec. Biol.* **66**, 403–421 (1972).
²³ Bernstein, H. J. *et al.* *Cryonet—A Network of Intelligent Remote Graphics Terminals. Second Annual AEC Scientific Computer Information Exchange Meetings, Proceedings of the Technical Program* (1974) 148–158 BNL-18803 (Brookhaven National Laboratory, New York, 1974).
²⁴ Obendorf, S. K., Carrell, H. L. & Glusker, J. P. *Acta crystallogr.* **B30**, 1408–1411 (1974).
²⁵ Jones, A. & Neidle, S. *Acta crystallogr.* **B31**, 1324–1334 (1975).
²⁶ Arnott, S., Hukins, D. W. L., Dover, S. D., Fuller, W. & Hodgson, A. R. *J. molec. Biol.* **81**, 107–122 (1973).
²⁷ Sobell, H. M., Tsai, C. C., Gilbert, S. G., Jain, S. C. & Sakore, T. D. *Proc. natn. Acad. Sci. U.S.A.* **73**, 3068–3072 (1976).
²⁸ Schmechel, D. E. V. & Crothers, D. M. *Biopolymers* **10**, 465–480 (1971).

Orientation of cell-surface antigens in the lipid bilayer of lymphocyte plasma membrane

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Human HLA-A, B, C and Ia antigens were labelled by lactoperoxidase-catalysed iodination of the inner surface of lymphocyte plasma membrane and thus are transmembrane proteins. In contrast, membrane-bound human IgM and mouse IgM, IgD and Thy-1 antigen were not labelled on the inner membrane surface.

AN important role of the plasma membrane is to transmit biochemical signals generated by ligand–receptor interaction at the cell surface across the lipid bilayer and into the cell¹. The elucidation of the mechanisms of transmembrane informational transfer depends ultimately on the designation of the orientation of the membrane proteins in the bilayer. In this respect the transmembrane proteins are especially favoured candidates since they are exposed on both the outer and inner (cytoplasmic) membrane faces. Thus, this orientation has apparently been universally adopted by membrane transport proteins^{2,3}. It is, however, evident that transmembrane proteins are not a prerequisite for signal transmission and that other mechanisms also exist. For instance, the biological effects of certain outer cell surface hormone receptors are believed to be mediated by interaction with cytoplasmically disposed adenylate cyclases⁴.

In view of the apparent functional importance of transmembrane proteins, there are strong incentives to develop methods for their identification. One possible way is by comparing the patterns produced by covalent labelling of the outer and inner surfaces of cells or plasma membranes^{5,6}. Application of this approach to erythrocytes^{7–11} has demonstrated that both of the major erythrocyte glycoproteins, glycophorin and band 3, are transmembrane molecules. The results of these studies have led to the proposals that all membrane glycoproteins have a transmembrane orientation, that the non-glycosylated proteins are exposed on the inner surface only and that no protein is present exclusively on the outer surface unless it is associated with a glycoprotein which spans the lipid bilayer^{12,13}. The notable successes that have been achieved with erythrocytes are primarily due to two factors—the lack of intracellular membranes and the ready availability of sealed ‘inside-out’ membrane vesicles for labelling the inner membrane surface^{7,8}. Several proteins in various other systems have also been shown to be transmembrane—for example, (Na⁺, K⁺)-dependent ATPase¹⁴, intestinal brush border aminopeptidase¹⁵, cytochrome *c* oxidase¹⁶, Semliki forest virus coat protein¹⁷ and rhodopsin¹⁸. No generally applicable method for their detection has, however, emerged with the result that knowledge of the orientation of membrane proteins of nucleated cells has proceeded slowly.

Designation of transmembrane proteins

We describe here a method for defining the transmembrane proteins of lymphocyte plasma membrane. Lymphocytes were chosen for study because their surface structure regulates many

biological effects (for example, activation by antigen, ‘patching’ and ‘capping’ of surface receptors) and includes many antigens of known specificity (for example, Ia and Thy-1 antigens, IgM and IgD). Also, well-characterised preparations of the surface membrane are available¹⁹. The approach adopted for designating the orientation of membrane proteins relied on lactoperoxidase-catalysed iodination for covalent labelling, and the availability of inside-out membrane vesicles that were sealed to lactoperoxidase. Lactoperoxidase-catalysed iodination was selected in preference to other techniques because the conditions favouring vectorial labelling have been clearly defined²⁰. In particular, the free iodide concentration was adjusted to less than 10^{−6} M to promote iodination via an impermeable enzyme-activated iodine complex²⁰. The procedure suffers, however, from a fairly high degree of selectivity in that only proteins with exposed tyrosine and possibly histidine residues are labelled. A method for the preparation of inside-out vesicles of pig lymphocyte plasma membrane has been described previously²¹. Although the vesicles were permeable to trypsin and concanavalin A, a subpopulation of vesicles has been selected by dextran density gradient centrifugation that were sealed to dextran of 10,000 molecular weight and also, by implication, to lactoperoxidase (molecular weight 78,000). Lactoperoxidase-catalysed iodination of these vesicles followed by affinity chromatography on concanavalin A–Sephacrose gave a different pattern of labelled polypeptides than that obtained with viable pig lymphocytes²². At least eight labelled polypeptides of each sample comigrated on polyacrylamide gel electrophoresis in sodium dodecylsulphate and were, consequently, judged to have a transmembrane orientation. A disadvantage of this study was the inability to equate any defined surface component with a particular iodinated polypeptide. This problem has been circumvented by using antisera against defined surface components to precipitate the homologous antigen from ¹²⁵I-labelled, inside-out membrane vesicles after solubilisation in sodium deoxycholate. The orientation of the following cell surface antigens have been analysed using this approach: the HLA-A, B, C and Ia antigens, and IgM of cultured human lymphoblastoid BRI 8 cells, as well as the Thy-1 antigen of mouse thymus cells, and IgM and IgD of mouse spleen cells.

Cell surface antigens

The choice of cell-surface antigens for study was determined by the availability of cells and specific antisera. The human B-lymphoblastoid cell line BRI 8 was chosen because it represents a well-documented source of HLA-A, B, C and Ia antigens and membrane-bound IgM^{23,24}. Mouse thymus and spleen cells have the advantage of being a relatively rich source of Thy-1 antigen²⁵ and membrane-bound IgM and IgD²⁶ respectively. Goat anti-human β_2 -microglobulin serum raised by immunisation with purified urinary β_2 -microglobulin was used as a ligand for the human HLA-A, B and C antigens. This choice is based on the observation that β_2 -microglobulin is non-covalently associated with the products of the HLA-A, B and C genes on the cell surface²⁷. The antiserum was shown to be monospecific on the

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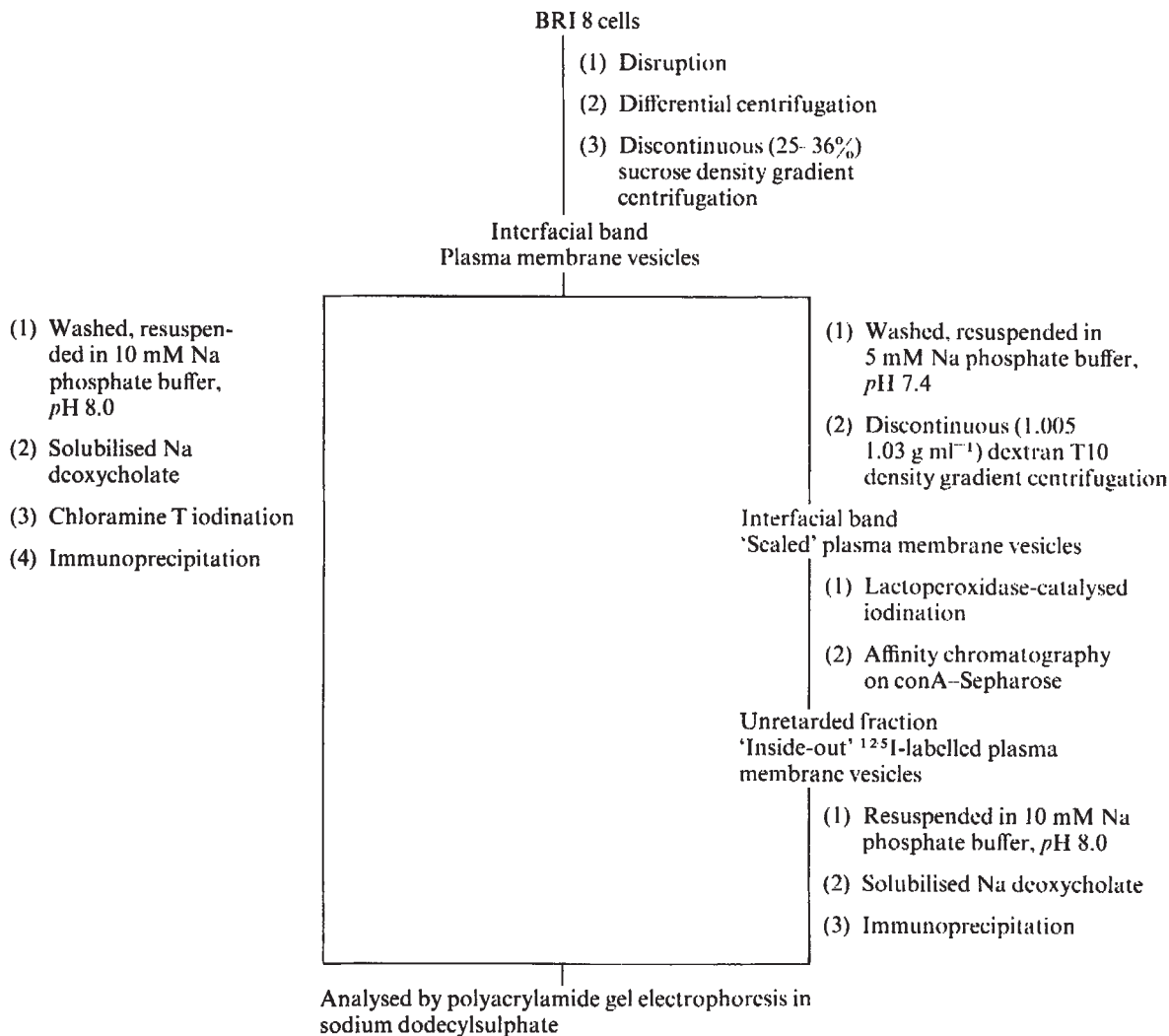


Fig. 1 Summary of experimental procedure for designating orientation of lymphocyte surface antigens. The procedure is shown for BRI 8 cells; an identical procedure was used for mouse spleen and thymus cells. The cells (about 80% viable; 5×10^7 cells ml⁻¹) were broken using the Stansted cell disruptor (model A0 612; disrupting valve 516; air pressure applied to disrupting valve was 55.2 kPa for BRI 8 and 250 kPa for mouse thymus and spleen cells). The plasma membrane fraction was separated as previously described^{19,21,23}, washed free of sucrose and resuspended in 5 mM Na phosphate buffer, pH 7.4. Sealed vesicles were separated by centrifugation on discontinuous gradients of Dextran T10 (Pharmacia) for 2 h at 40,000 r.p.m. in a Beckman SW41 rotor⁸. The vesicles that accumulated at the interface between 1.005 and 1.03 g ml⁻¹ were impermeable to dextran of 10,000 molecular weight. This fraction represented at least 25% of BRI 8 plasma membrane but was no more than 10% of the membrane preparations from mouse spleen or thymus cells. The sealed vesicles (2–3 mg of membrane protein in 1 ml of 5 mM Na phosphate buffer, pH 7.4) were iodinated by adding 1 mCi Na¹²⁵I, 20 μ g lactoperoxidase, 2.3 units glucose oxidase and glucose to a final concentration of 20 mM. After 15 min at 20 °C the reaction was terminated by adding 40 volumes of 5 mM Na phosphate buffer, pH 7.4, at 4 °C. The iodinated membrane was recovered by sedimentation at 75,000g for 30 min and inside-out vesicles were separated as described previously using concanavalin A-Sepharose 4B²¹. The ¹²⁵I-labelled inside-out membrane vesicles (about 1 mg of protein) were solubilised at 4 °C by addition of Na deoxycholate [10% (w/v) in 10 mM Tris-HCl buffer, pH 8.2] to a final concentration of 2% (w/v). After 30 min the solution was centrifuged at 100,000g for 1 h. Plasma membranes from BRI 8, mouse spleen and mouse thymus cells for control immunoprecipitations (about 1 mg of each) were solubilised in 1 ml of 2% (w/v) Na deoxycholate and non-vectorially labelled with Na¹²⁵I (1 mCi) by using Chloramine T (10 μ g)²⁰.

basis of being inhibited completely by an authentic sample of β_2 -microglobulin, and having no detectable reactivity with erythrocytes and the lymphoblastoid cell line 'Daudi' which lack HLA-A, B and C antigens. A rabbit antiserum raised by hyperimmunisation with a glycoprotein fraction from BRI 8 plasma membrane was used to precipitate the HLA-Ia antigens—according to various criteria this antiserum recognised the human counterparts of the mouse Ia antigens^{24,28}. Mouse thymocyte Thy-1 was precipitated by a xenogeneic cross-reacting antiserum raised against rat brain Thy-1^{25,29} (from A. F. Williams). IgM from BRI 8 cells was precipitated by a commercial sample (Organon) of goat anti-(human IgM) serum, and mouse spleen membrane IgM and IgD were precipitated using an antiserum raised against mouse serum Ig (from M. C. Raff).

Numerous studies have shown that each of the above antigens is exposed on the cell surface. This disposition was confirmed for the cells chosen for study by using fluorescein-labelled anti-(Ig) sera to

reveal antigen-specific antibody bound to the cell surface in the fluorescence activated cell sorter. Thus, all BRI 8 cells were stained by anti-(β_2 -microglobulin), anti-(HLA-Ia) and anti-(IgM) sera relative to control cells which had not been incubated with specific antibody. Similar results were found with the anti-(Thy-1) serum on mouse thymus cells, whereas anti-(mouse Ig) serum stained about 50% of mouse spleen cells.

¹²⁵I-labelled inside-out plasma membrane vesicles

The preparation of iodinated inside-out plasma membrane vesicles from the various cell-types is summarised in Fig. 1. The initial purified plasma membrane preparation was not significantly contaminated by other subcellular fractions¹⁹. Previous results indicated that it represented a mixture of right-side-out and inside-out vesicles that were permeable to certain macromolecules²¹. Fractionation of the vesicles on a discontinuous density gradient

of dextran T10, in a manner similar to that used for preparing sealed erythrocyte vesicles⁸, yielded a population which by implication was impermeable to lactoperoxidase and which, thus, permitted vectorial labelling. The proportion of vesicles sealed to dextran T10 varied with the cell type and was not easily manipulated. Iodination of these vesicles was followed by fractionation on concanavalin A-Sepharose 4B (ref. 21) to separate the inside-out vesicles.

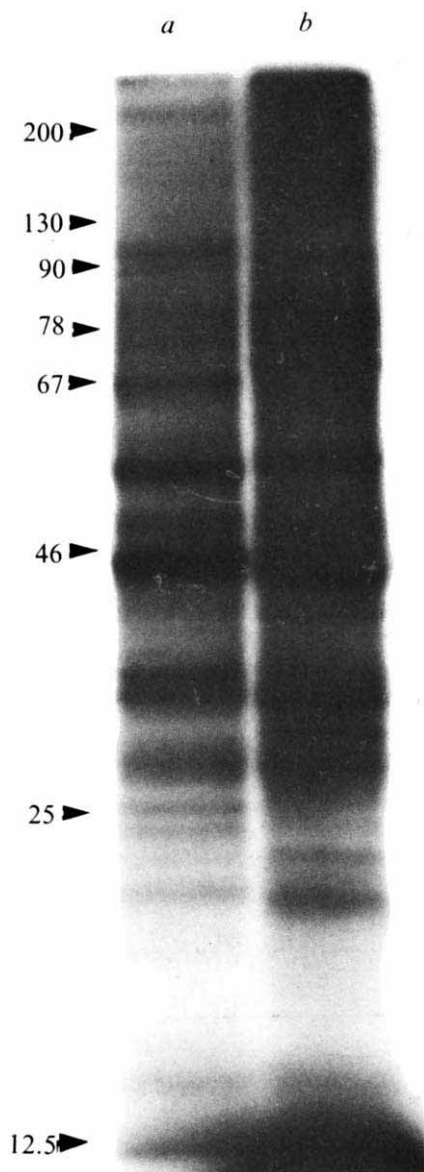


Fig. 2 Comparison of polypeptides labelled by lactoperoxidase-catalysed iodination of *a*, whole BRI 8 cells and *b*, inside-out plasma membrane vesicles. BRI 8 cells [10^7 in 100 μ l of phosphate buffered saline (PBS, 10 mM Na phosphate buffer 0.15 M NaCl, pH 7.4)], were iodinated by adding 300 μ Ci of Na 125 I, 20 μ g ml $^{-1}$ of lactoperoxidase, 2.3 units ml $^{-1}$ glucose oxidase and glucose to a final concentration of 20 mM. The reaction was allowed to proceed for 15 min at 20 °C and was terminated by the addition of 100 volumes of PBS. The cells were washed twice and finally resuspended in 1 ml of sodium dodecyl sulphate (SDS) sample buffer [2% (w/v) SDS, 10% (w/v) glycerol, 0.01 M dithiothreitol and 0.02% (w/v) bromophenol blue]. 125 I-labelled inside-out plasma membrane vesicles were prepared from BRI 8 cells as described in Fig. 1. They were sedimented by centrifugation at 75,000g for 30 min and resuspended in SDS sample buffer. Samples were boiled for 2 min and analysed by polyacrylamide gel electrophoresis in SDS using the Tris-glycine discontinuous buffer system of Laemmli³¹. Iodinated polypeptides were revealed by autoradiography. Molecular weights (given in thousands) were calculated by reference to the mobilities of standard proteins: myosin (200), β -galactosidase (130), phosphorylase (90), transferrin (78), bovine serum albumin (67), ovalbumin (46), Ig L-chain (25) and cytochrome C (12.5).

Figure 2 shows representative patterns of the iodinated polypeptides of 125 I-labelled whole BRI 8 cells (the outer surface) and of 125 I-labelled inside-out plasma membrane vesicles (the inner surface), as revealed by polyacrylamide gel electrophoresis in sodium dodecylsulphate. The clear differences between the patterns inspires confidence in the efficacy of the overall procedure. The samples contained more than 10 co-migrating polypeptides which on the basis of this criterion have a transmembrane orientation. Similar results have been obtained for pig lymphocytes²² as well as mouse thymus and spleen cells.

Immunoprecipitations

Immunoprecipitation analysis of the 125 I-labelled inside-out vesicles after solubilisation in sodium deoxycholate was undertaken to determine whether any of the inner-surface iodinated polypeptide chains could be equated with known cell surface antigens. A positive result would indicate that the antigen in question was exposed on both faces of the lipid bilayer and was, thus, a transmembrane protein. Each of the cell surface antigens chosen for study was also shown to be a component of the purified plasma membrane by immunoprecipitation from solubilised membrane that had been labelled non-vectorially by iodination using Chloramine T³⁰. The results for the various antigens are summarised in Fig. 3.

The HLA-A, B and C gene products are glycosylated polypeptides of 43,000 molecular weight²³. Immunoprecipitation of the 125 I-labelled inside-out membrane vesicles with the anti-(β_2 -microglobulin) serum gave one band only of molecular weight 43,000 (Fig. 3b), whose position was identical with that of the band of the control precipitate from the non-vectorially iodinated plasma membrane (Fig. 3a). If lactoperoxidase-catalysed iodination of the inside-out vesicles labels exposed tyrosine residues on the inner membrane surface only, then this result indicates that the HLA-A, B and C polypeptides span the lipid bilayer. The β_2 -microglobulin component of the HLA-A, B and C antigens was not detected because at the polyacrylamide gel concentration used (7.5%) β_2 -microglobulin was not resolved from the dye front and was run off the gel together with the radioactive iodide.

Similar results were obtained for the HLA-Ia antigens. These antigens comprise two non-covalently associated glycosylated polypeptides of 28,000 and 33,000 molecular weight²⁴. Figure 3c shows that the plasma membrane contained both polypeptides and Fig. 3d indicates that both are exposed on the inner membrane surface.

In contrast, neither the μ nor L chains of human IgM were labelled in the inside-out vesicles (Fig. 3f), although Fig. 3e indicates that the plasma membrane contained IgM and that both chains were iodinated when the Chloramine-T procedure was used. An identical result was obtained for mouse spleen cell Ig. Thus, the μ , δ and L chains were present in the plasma membrane (Fig. 3g), but were not labelled in the inside-out membrane vesicles (Fig. 3h). Similarly, Thy-1 antigen (molecular weight about 25,000³³) was a component of the thymus plasma membrane (Fig. 3i), but was not labelled in the inside-out vesicles (Fig. 3j).

Conclusions

The significance of the results depends on the subpopulation of plasma membrane vesicles used for the labelling studies being correctly designated as inside-out and sealed to lactoperoxidase. This designation is supported by two experiments. First, the failure to label certain known membrane components by lactoperoxidase-catalysed iodination (Fig. 3) argues strongly against the vesicles being leaky to lactoperoxidase. Second, the different labelling patterns given by intact cells and by the membrane vesicles (Fig. 2) argues that the outer surfaces of the cells and membrane vesicles possess different structures which is consistent with a different orientation. If these interpretations are correct then the prescribed method is applicable to the identification of lymphocyte transmembrane proteins as well as providing the means to probe the structure of the plasma membrane's inner surface. It seems likely that the approach will be generally applicable to a variety of cell types, especially since inversion of plasma orientation may be a general feature of cell breakage³⁴.

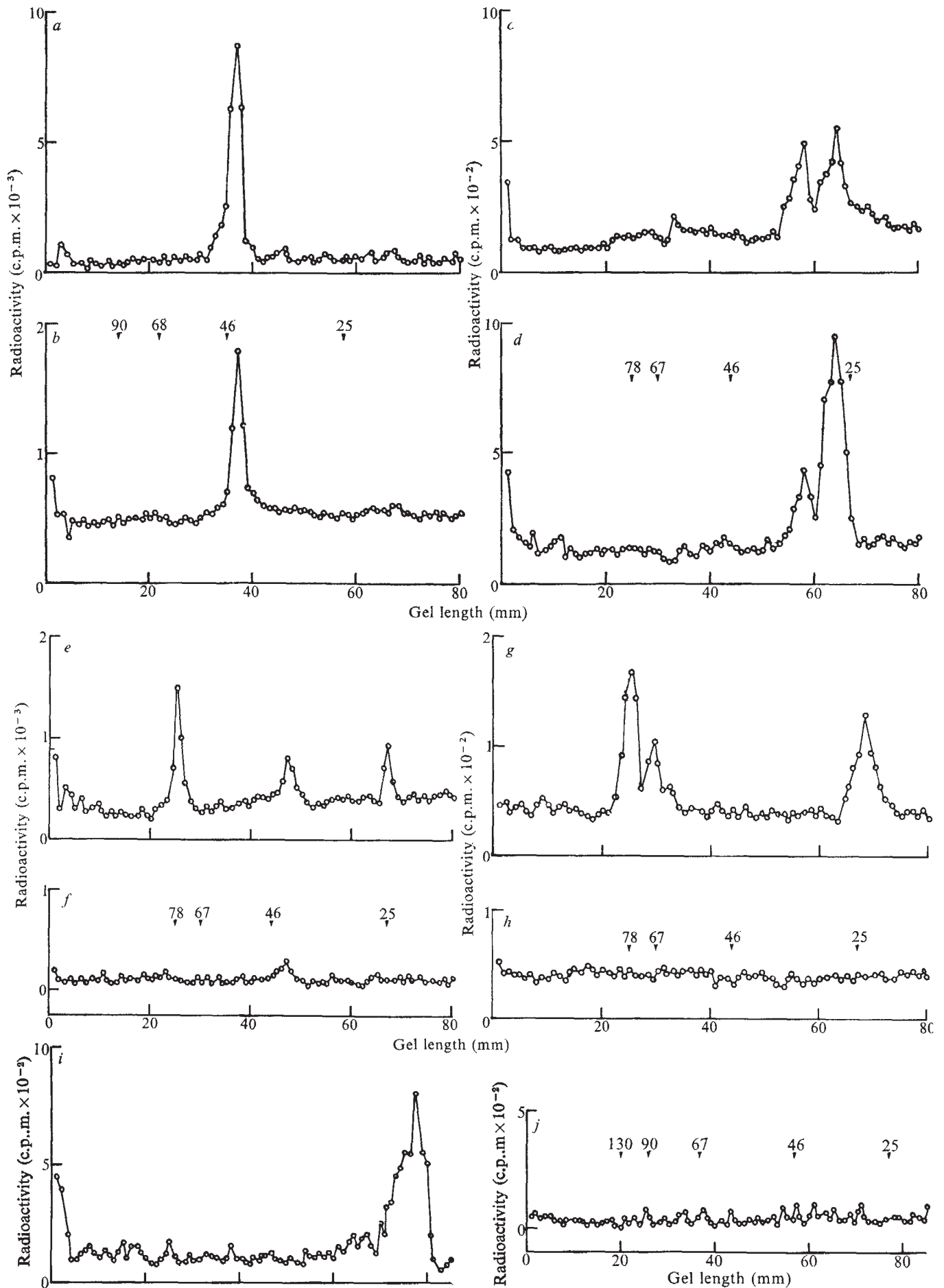


Fig. 3 For description see opposite

Fig. 3 Polyacrylamide gel electrophoresis patterns in SDS of immunoprecipitates prepared from ^{125}I -labelled inside-out membrane vesicles with *b*, anti-(human β_2 -microglobulin); *d*, anti-(HLA-Ia); *f*, anti-(human IgM); *h*, anti-(mouse Ig) and *j*, anti-(Thy-1) sera. These patterns should be compared with those obtained from the non-vectorially iodinated plasma membrane with *a*, anti-(β_2 -microglobulin); *c*, anti-(HLA-Ia); *e*, anti-(human IgM); *g*, anti-(mouse Ig) and *i*, anti-(Thy-1) sera. The preparation of ^{125}I -labelled inside-out membrane vesicles and ^{125}I -labelled plasma membrane is described in Fig. 1. Three methods of immunoprecipitation were used depending on the quantity of antiserum available. First, the Ig fractions of the anti-(β_2 -microglobulin), anti-(HLA-Ia), anti-(mouse Ig) and normal rabbit serum were coupled to cyanogen bromide activated Sepharose CL-4B at a concentration of 10 mg of protein per ml of gel sediment. Before interaction with specific antibody the deoxycholate-solubilised ^{125}I -labelled membranes (Fig. 1) were depleted of non-specifically bound protein by incubation for 30 min at 20 °C on a 5 ml column of normal rabbit Ig-Sepharose CL-4B. Unadsorbed protein was eluted with 0.5% (w/v) Na deoxycholate. The eluate was incubated for 30 min at 20 °C on 5 ml columns of immune Ig-Sepharose CL-4B that were subsequently washed with 0.5% (w/v) Na deoxycholate. The adsorbed material was eluted with 2% (w/v) SDS in 8 M urea, precipitated with ethanol (final concentration 66%) for 48 h at -20 °C, solubilised in SDS sample buffer and analysed by polyacrylamide gel electrophoresis in SDS as described above. After electrophoresis and staining with Coomassie blue the gels were dried, divided into 1 mm slices and counted in a Packard gamma counter. Second, *Staphylococcus aureus* A protein attached to Sepharose CL-4B was used as the absorbent for the anti-(human IgM) serum. The protein prepared as previously described³² was coupled to Sepharose CL-4B (1 mg of protein per ml of gel sediment). The deoxycholate-solubilised ^{125}I -labelled BRI 8 membranes were pre-adsorbed with normal rabbit Ig-Sepharose CL-4B as described above. The eluate was incubated at 37 °C with 100 μl of anti-(human IgM) serum for 1 h followed by 12 h at 4 °C and then incubated for 30 min at 20 °C with the *Staph* A-Sepharose CL-4B. Unadsorbed material was eluted with 0.5% (w/v) Na deoxycholate. The bound material was displaced with 2% (w/v) SDS in 8 M urea and treated as previously described. Third, a direct precipitation system was used for the mouse thymus Thy-1 antigen. Deoxycholate-solubilised ^{125}I -labelled membrane (300 μl) was incubated at 37 °C for 30 min with 30 μl of anti-(Thy-1) serum, for a similar period with 50 μl of horse anti-(rabbit Ig) and then cooled to 4 °C for 12 h. The resulting precipitate was washed four times with 0.5% (w/v) Na deoxycholate at 4 °C, solubilised in 2% (w/v) SDS in 8 M urea and analysed as above.

The major limitation of the method concerns the selectivity of iodination which is restricted to exposed tyrosine residues. As a result, lack of labelling may reflect lack of an accessible tyrosine residue rather than absence of the polypeptide chain *per se*.

The plasma membrane of the human B-lymphoblastoid cell line BRI 8 contained at least 10 transmembrane proteins as assessed by the comigration of polypeptide chains which were iodinated on both the outer surface of intact cells and of inside-out membrane vesicles (Fig. 2). Similar results have been obtained with pig lymphocytes²². In this case the majority of the transmembrane proteins were further identified as glycoproteins on the basis of specific adsorption to *Lens culinaris* lectin-Sepharose²². A preferential disposition of glycoproteins to a transmembrane orientation is in accord with models of plasma membrane structure based on erythrocytes^{12,13}.

Direct evidence was obtained that the polymorphic, 43,000 molecular weight polypeptide chain of the HLA-A, B and C antigens spans the lipid bilayer. This conclusion is in accord with the orientation suggested previously on the basis of the amino acid composition of enzymatic cleavage products³⁵. It is also concluded that both of the polypeptides (28,000 and 33,000 molecular weight) which comprise the HLA-Ia antigens are transmembrane.

The significance of the lack of labelling of Thy-1 antigen and membrane-bound Ig in the inside-out vesicles is equivocal because of the selectivity of the labelling procedure. Other arguments may, however, be invoked in favour of the view that the lack of labelling of IgM is a true reflection of its orientation. Thus, membrane-bound IgM binds very much less detergent than typical integral membrane proteins including Thy-1 and HLA-A, B and C and Ia antigens^{24,33,36}. This difference in behaviour is consistent with the proposal that IgM dips much less extensively into the bilayer than known transmembrane proteins. Also, if the μ -chain of membrane-bound IgM spans the membrane it should be iodinated,

assuming its structure is identical with that of secreted IgM which has tyrosine as its C-terminal amino acid residue³⁷.

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- ¹ Crumpton, M. J., Allan, D., Auger, J., Green, N. M. & Maino, V. C. *Phil. Trans. R. Soc.* **B272**, 173-180 (1975).
- ² Singer, S. J. *A. Rev. Biochem.* **43**, 805-833 (1974).
- ³ Guidotti, G. *Trends biochem. Sci.* **1**, 11-13 (1976).
- ⁴ Greaves, M. F. *Nature* **265**, 681-683 (1977).
- ⁵ Bretscher, M. S. *J. molec. Biol.* **59**, 351-357 (1971).
- ⁶ Carraway, K. L. *Biochim. biophys. Acta* **415**, 379-410 (1975).
- ⁷ Steck, T. L. *J. Cell Biol.* **62**, 1-19 (1974).
- ⁸ Steck, T. L. *Meth. Membr. Biol.* **2**, 245-281 (1974).
- ⁹ Mueller, T. J. & Morrison, M. *Biochemistry* **14**, 5512-5516 (1975).
- ¹⁰ Reichstein, E. & Blostein, R. *J. biol. Chem.* **250**, 6256-6263 (1975).
- ¹¹ Boxer, D. H., Jenkins, R. E. & Tanner, M. J. A. *Biochem. J.* **137**, 531-534 (1974).
- ¹² Bretscher, M. S. & Raff, M. C. *Nature* **258**, 43-49 (1975).
- ¹³ Rothman, J. E. & Lenard, J. *Science* **195**, 743-753 (1977).
- ¹⁴ Kyte, J. *J. biol. Chem.* **250**, 7443-7449 (1975).
- ¹⁵ Louvard, D., Semeriva, M. & Maroux, S. *J. molec. Biol.* **106**, 1023-1035 (1976).
- ¹⁶ Hackenbrock, C. R. & Hammon, K. M. *J. biol. Chem.* **250**, 9185-9197 (1975).
- ¹⁷ Garoff, H. & Simons, K. *Proc. natn. Acad. Sci. U.S.A.* **71**, 3988-3992 (1974).
- ¹⁸ Blaurock, A. E. *J. molec. Biol.* **93**, 139-158 (1975).
- ¹⁹ Crumpton, M. J. & Snary, D. *Contemp. Top. molec. Immun.* **3**, 27-56 (1974).
- ²⁰ Hubbard, A. L. & Cohn, Z. A. in *Biochemical Analyses of Membranes* (ed. Maddy, A. H.) 427-501 (Chapman and Hall, London, 1976).
- ²¹ Walsh, F. S., Barber, B. H. & Crumpton, M. J. *Biochemistry* **15**, 3557-3563 (1976).
- ²² Walsh, F. S., Barber, B. H. & Crumpton, M. J. *Biochem. Soc. Trans.* (in the press).
- ²³ Bridgen, J. *et al. Nature* **261**, 200-205 (1976).
- ²⁴ Snary, D., Barnstable, C. J., Bodmer, W. F., Goodfellow, P. N. & Crumpton, M. J. *Scand. J. Immun.* **6**, 439-452 (1977).
- ²⁵ Trowbridge, J. S., Weissman, I. L. & Bevan, M. J. *Nature* **256**, 652-654 (1975).
- ²⁶ Goding, J. W. & Layton, J. E. *J. exp. Med.* **144**, 852-857 (1976).
- ²⁷ Tanigaki, N. & Pressman, D. *Transplant. Rev.* **21**, 15-34 (1974).
- ²⁸ Barnstable, C. J. *et al. Cold Spring Harb. Symp. quant. Biol.* **41**, 443-455 (1977).
- ²⁹ Williams, A. F. *Contemp. Top. molec. Immun.* **6**, 83-116 (1977).
- ³⁰ Greenwood, F. C., Hunter, W. M. & Glover, J. S. *Biochem. J.* **89**, 114-123 (1963).
- ³¹ Laemmli, U. K. *Nature* **227**, 680-685 (1970).
- ³² Sjöquist, J., Meloun, B. & Hjelm, H. *Eur. J. Biochem.* **29**, 572-578 (1972).
- ³³ Barclay, A. N., Letarte-Muirhead, M., Williams, A. F. & Faulkes, R. A. *Nature* **263**, 563-567 (1976).
- ³⁴ Bennett, V. & Cuatrecasas, P. *Biochim. biophys. Acta* **311**, 362-380 (1973).
- ³⁵ Springer, T. A. & Strominger, J. L. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2481-2485 (1976).
- ³⁶ Melcher, U. & Uhr, J. W. *Biochemistry* **16**, 145-152 (1977).
- ³⁷ Putnam, F. W., Florent, G., Paul, C., Shinoda, T. & Shimizu, A. *Science* **182**, 287-291 (1973).

letters to nature

Possible fast variability of the nucleus of Cen A at 13.5 mm

INFORMATION about short time scale events in extragalactic objects can have very important implications for the theoretical interpretation of the physical mechanisms involved. Such data are only obtained by using time-consuming observing schedules and methods, and are not

usually carried out at radio observatories. This kind of information is thus generally lacking. We report here a study of the nucleus of Cen A (NGC 5128) at a wavelength of 13.5 mm. Possible fast variability was observed.

Observations in the direction of the infrared 'hot-spot' of NGC 5128 (ref. 1) have been made at centimetre and millimetre wavelengths²⁻⁴. Kellermann⁵ has suggested an important intraday change of flux, based on few observations taken at 3.4 and 9.5 mm. Fogarty and Schuch⁶ found no variability using observations obtained monthly at

13.5 mm, in 1974. X-ray emission from the nucleus of NGC 5128 was identified by Tucker *et al.*⁵, and important variability has been claimed by other workers at X-ray wavelengths^{6,7}.

We performed 37 observations of the nucleus of NGC 5128 at $\lambda=13.5$ mm from July 1976 to March 1977, which included two periods of 15 d sequential patrol (26 July to 10 August, and 27 November to 13 December, in 1976). The observations were carried out using a 45-foot dish at Itapetinga Radio Observatory, Atibaia, Brazil. All observations were performed at the same sidereal time at the local meridian transit to avoid polarisation effects. The antenna beam at 13.5 mm is of about 4' arc, and observations were made using on-on and beamswitching techniques.

For comparison we observed Vir A in sequence, as a calibrator (for which we adopted 21.4 Jy). After 10 November 1976 we also observed the NE lobe of NGC 5128 (13 h 42 min 48 s, $-42^\circ 42' 11''$, 1950.0 coordinates). This position is situated nearly 2.5' arc NE of the nucleus such that there was only a weak beam overlap with less than about 3 dB of attenuation. This lobe was selected for comparison since close to it, in NE direction, it has been recently found some H II filamentary structures (ref. 8 and M. Dennefeld, personal communication). It corresponds to a structure identified at decimetric and metric wavelengths⁹⁻¹¹.

The measurements were normalised against Virgo A and are shown in Fig. 1. Nearly all observations were carried in the following sequence of 30 min integrations: Vir A, NE lobe, centre, Vir A. In some days the integration times were extended with 15 min extra observations. Sky attenuation was measured before and after the sequence, and the average was adopted for data correction. The error bars are $\pm 1\sigma$ referred to the worst 30-min integration measurement of the sequence. Larger error bars refer to days with bad weather, and the measurements are consequently less

reliable. In general both atmospheric and instrumental changes affected similarly all observations from a sequence.

Figure 1 suggest an 'event' (with flux reduction) in 22 November–5 December 1976, in the centre/Vir A and centre/NE lobe series of observations. This is not suggested in the NE lobe/Vir A series. Both centre/Vir A and NE lobe/Vir A data show reduced values in 11–12 November 76 and 10–11 March 77—and this is not seen in centre/NE lobe data.

It should be stressed that the observations of the centre and NE lobe were performed in nearly equal conditions, using the same corrections (elevation angle of about 70°). Vir A was observed in the opposite hemisphere at a lower elevation angle (about 45°), and the measurements suffered different corrections. An error in the estimate of the sky attenuation will not affect the relative measurement centre/NE lobe, but may affect Vir A corrected measurements, and thus centre/Vir A and NE lobe/Vir A comparisons. Thus the centre/NE lobe data are the most reliable for comparison purposes.

The coincidence in the 'event' occurrence both in the more reliable comparison centre/NE lobe and in centre/Vir A suggests that it might be of a real nature. On the other hand, due to the reasons discussed above, the reduction in centre/Vir A and NE lobe/Vir A in November 76 and March 77 could not be attributed necessarily to changes in Vir A.

The possible 'event' described here is relatively much better defined than Kellerman's³ 2 d data at 3.4 mm, which have also shown a flux reduction of nearly 30%. This reduction alone bring some complications for the interpretation. But we feel the data are still few, and the effect might be only a part of a larger more complex event.

Long term data seem to confirm that there are no large time scale variations in flux from the nucleus of Cen A

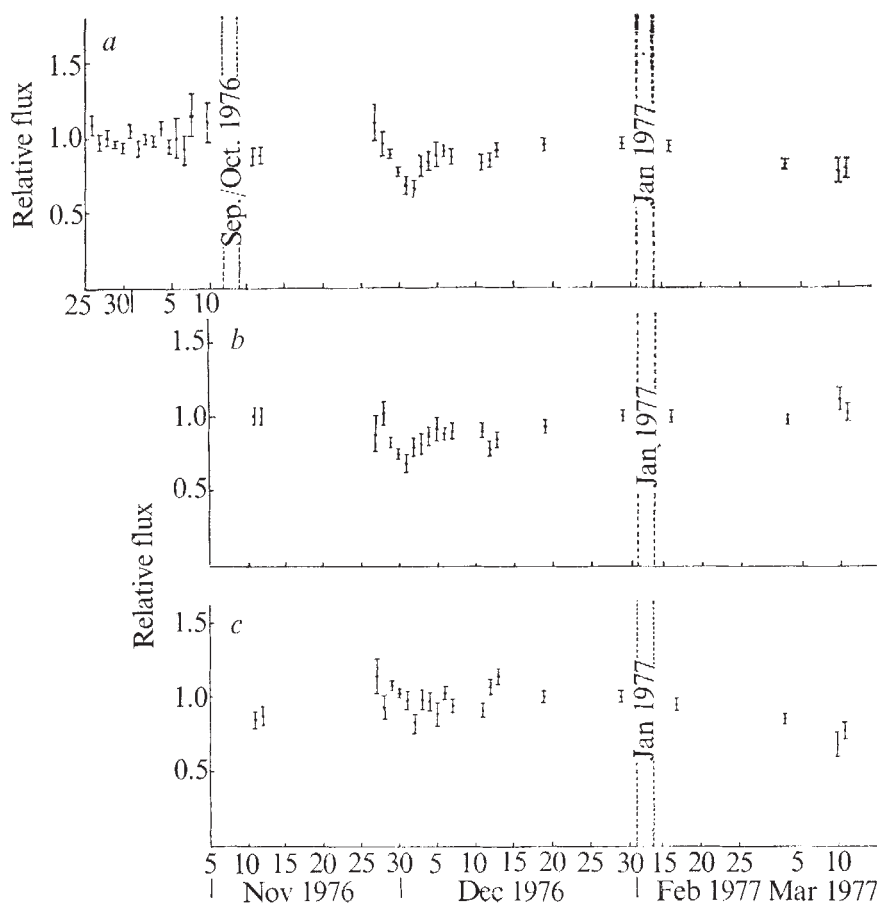


Fig. 1 Observations at 13.5 mm of the nucleus of Cen A in relation to Virgo A (a), of the nucleus of Cen A in relation to NE Lobe of Cen A (b), and of NE Lobe of Cen A in relation to Vir A (c). Ordinates are in relative units. Error bars correspond to standard errors of the mean.

(ref. 4). Short time scale events might happen within a few days, however. Complete description of the pattern will require daily observations over several months.

It should be noted that the previous map¹² of NGC5128 was derived with isotherms referred to the centre, for which variability was not taken into account. An improved version of the map at 13.5 mm is under investigation.

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Received 29 March; accepted 5 July 1977.

- ¹ Kunkel, W. E. & Bradt, H. V. *Astrophys. J.* **170**, L7-L10 (1971).
- ² Price, K. M. & Stull, M. A. *Nature* **245**, 83-89 (1973).
- ³ Kellermann, K. I. *Astrophys. J.* **194**, L135-L137 (1974).
- ⁴ Fogarty, W. G. & Schuch, N. J. *Nature* **254**, 124-125 (1975).
- ⁵ Tucker, W., Kellogg, E., Gursky, H., Giacconi, R. & Tananbaum, H. *Astrophys. J.* **180**, 715-724 (1973).
- ⁶ Winckler, P. F. & White, A. E. *Astrophys. J.* **199**, L139-L142 (1975).
- ⁷ Fabian, A. C., Maccagni, D., Rees, M. J. & Stoeger, W. R. *Nature* **260**, 683-685 (1976).
- ⁸ Blanco, V. M., Graham, J. A., Lasker, B. M. & Osmer, P. S. *Astrophys. J.* **198**, L63-L64 (1975).
- ⁹ Cooper, B. F. C., Price, R. M. & Cole, D. J. *Austr. J. Phys.* **18**, 589-625 (1965).
- ¹⁰ Lockhart, I. A. & Sheridan, K. V. *Proc. ASA* **1**, 344-345 (1970).
- ¹¹ Slee, O. B. & Sheridan, K. V. *Proc. ASA-2* **6**, 1 (1975).
- ¹² Kaufmann, P., Scalise, Jr, E., Marques dos Santos, P. & Fogarty W. G. *Mon. Not. R. astr. Soc.* **169**, 15p-17p (1974).

Pi 1-2 magnetic field pulsations on dayside cleft field lines

TYPE Pi 1-2 magnetic field pulsations, $T = 1-150$ s, have a broad-band, noise-like character and a tendency to occur during active aurorae and magnetospheric substorms^{1,2}. It is well-known that type Pi 1-2 magnetic field pulsations observed in the night-morning sector during magnetospheric substorms maximise in amplitudes along the auroral oval^{1,2}. But the relation of the Pi 1-2 activity to the day sector of the auroral oval has not been clear. This relation is of particular interest because the day sector of the auroral oval seems to coincide approximately with the feet of the dayside cleft field lines; the precise relation between the dayside cleft and the dayside auroral oval, however, has not yet been established (S. I. Akasofu, personal communication). Dayside cleft field lines usually terminate between the geomagnetic latitudes of 75° and 80°. We show here that intense Pi 1-2 activity appeared at College on two occasions when the cleft came down to the College latitude, 64.7° N, at times when College was in the midday sector. Further, it is shown that an intense flux of particle precipitation into the dayside ionosphere occurred on the second occasion, 4 August 1972.

A direct comparison between Pi 1-2 activity as observed at Bar I (70.3°N, 257°E, geom.) and College (64.7°N, 257°E) on 26-27 July 1969, and observations of the dayside cleft by satellite³ is shown in Fig. 1. In the interval 2000-0100 UT, which is local midday at Bar I and College, the cleft moved towards the equator, approaching 66° at 0100 UT. Pi 1-2 activity was most intense at Bar I between 2300 and 0100 UT when Bar I was on cleft lines. Pi 1-2 was most prominent at College between 0100 and 0200 UT, but was not as intense as at Bar I (maximum amplitudes near 0.02 Hz were 16 nT at Bar I and 6 nT at College). This behaviour is consistent with a Pi 1-2 origin on cleft lines.

The cleft tends to move to smaller L values ($L = \text{McIlwain's parameter}$; the invariant latitude ($\Lambda_0 = \cos^{-1}(1/L)^{1/2}$), with increasing values of the AE index³. From this, we would expect Pi

activity to be seen at College in midday hours preferentially at times of high AE (or Kp) if the midday Pi 1-2 activity does maximise on cleft lines. To check this, we looked at College Pi data^{4,5} and found that Pi occurred in midday hours at College on about 30 d in 1966 and 1967 with Kp values ranging from 4 to 9. In contrast, Pi activity is prominent at College in night hours for Kp from 2 to 5. These characteristics seem consistent with a Pi oval that coincides approximately with the cleft lines on the day side.

On 4 August 1972, at 2240 UT, the magnetopause moved inward past Explorer 45, then at $L = 5.2$ and at 1435 MLT⁶.

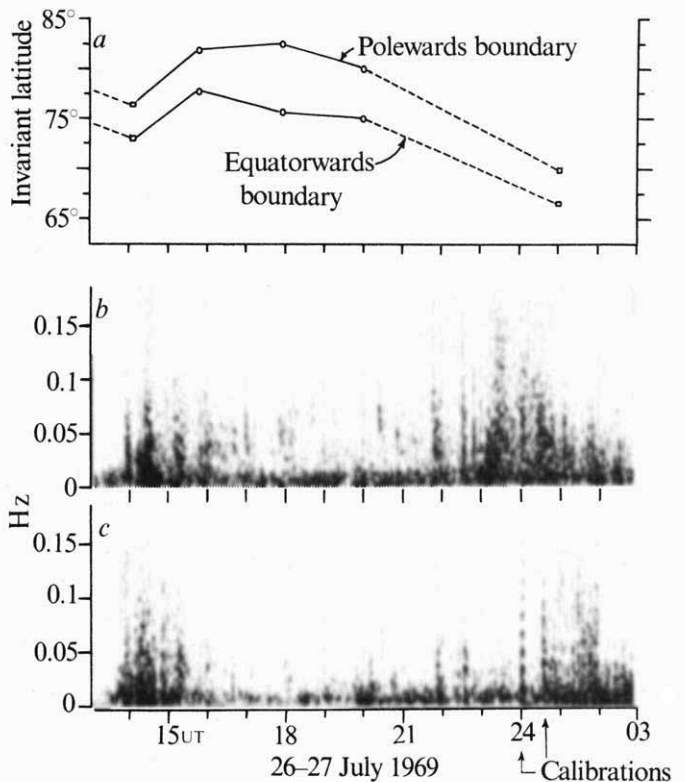


Fig. 1 Appearance of type Pi 1-2 pulsations at b, Bar I and c, College 2300-0200 UT when dayside cleft lines were in the vicinity of those sites. a, from Yasuhara and Akasofu ref. 3.

An induction magnetometer operating near Chatanika, $L = 5.6$, 40 km north of College, registered very strong Pi 1-2 activity at that time. The activity actually exceeded the record range of the tape recorder (30 nT at 0.02 Hz). Sonagrams show that the pulsations had the broad-band form that characterises Pi 1-2 activity. The Kp index was 9₀ at the time and AE reached 2065 nT.

Since the magnetopause moved inwards past $L = 5.2$ at 2240 UT, it evidently passed $L = 5.6$ somewhat earlier, hence Chatanika was on cleft lines for the duration of the strong Pi activity observed there. Direct Chatanika radar observations showed the cleft to be located near Chatanika from 2200-2300 UT on 4 August⁷.

The Chatanika radar⁸ was in operation at the time, and the data show that prominent E- and D-regions were formed⁹ (Fig. 2). The College 30 MHz riometer went off scale in this interval, the absorption exceeding 20 dB (ref. 11). These measurements show conclusively that a field-aligned plasma stream was present on the dayside cleft field lines. Using Chatanika radar electron densities and appropriate auroral ionosphere D-region collision frequencies, the absorption at Chatanika was estimated to be 21.4 dB at 30 MHz, which could be produced by 70 keV electrons with a minimum precipitation rate of 3×10^6 e cm⁻³ s⁻¹ ster⁻¹ (ref. 10). Similar estimates of precipitating particle energies during this period have been given by other investigators¹².

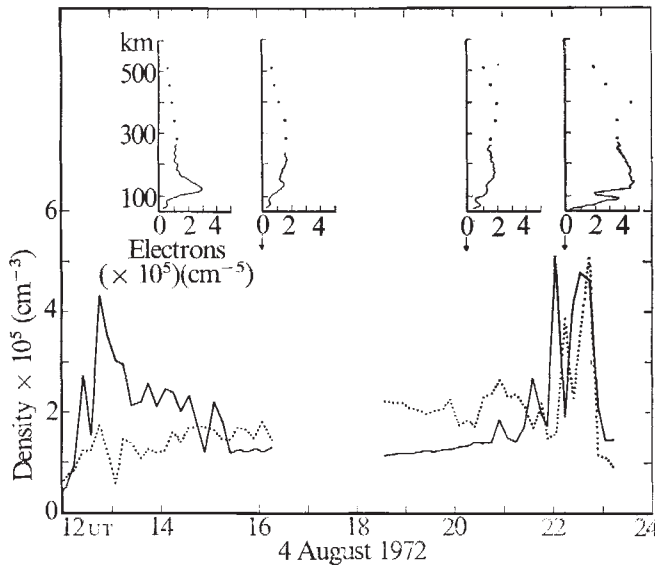


Fig. 2 Chatanika radar electron density data for 4 August 1972. Densities of up to $5 \times 10^5 \text{ cm}^{-3}$ were observed at the feet of dayside cleft field lines 2200–2300 UT (from Watkins *et al.*, ref. 7).

Since an intense field-aligned flux of electrons was present on the field lines where the Pi 1–2 pulsations were observed, it is possible to invoke the garden-hose overstability mechanism for the generation of the Pi 1–2. The garden-hose overstability is a consequence of field-aligned plasma streaming in the presence of magnetic mirroring, conditions that are met in the magnetosphere¹³. The plasma pressure becomes anisotropic with $P_{\parallel} > P_{\perp}$, the anisotropy increasing with increasing streaming speed, and this anisotropy generates Alfvén waves⁹. Thus particle energies of 1–100 keV are favourable for the generation of the unstable Alfvén waves through the garden-hose mechanism, and those energies were observed in the cleft region in the case of the 4 August 1972 activity⁸.

Observations of strong Pi 1–2 activity in the day sector may be useful for locating cleft field lines in experiments where supplementary knowledge of the cleft location is required. In recent years, the most usual ground-based technique for locating the cleft field lines in the ionosphere has been the ionosonde technique. Since magnetometer data are considerably easier to obtain than ionosonde, particularly on a continuous long-term basis, it is advisable to extend the present investigation to Pi 1–2 recording sites between 70° and 80°.

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- ¹ Akasofu, S.-I. *Polar and Magnetospheric Substorms* (Reidel, Dordrecht-Holland 1968).
- ² Saito, T. *Space Sci. Rev.* 22, 319–412 (1969).
- ³ Yasuhara, F. & Akasofu, S.-I. *J. geophys. Res.* 78, 7286–7291 (1973).
- ⁴ Heacock, R. R. & Hessler, V. P. *High Latitude Geophysical Data, Reports UAG-C 43 to UAG-C 47* (Geophysical Institute of the University of Alaska, 1966–1967).
- ⁵ Hessler, V. P. *High Latitude Geophysical Data, Reports UAG-C 43 to UAG-C 47*, (Geophysical Institute of the University of Alaska, 1966–1967).
- ⁶ Hoffman, R. A. *et al. J. geophys. Res.* 80, 4287–4296 (1975).
- ⁷ Wedde, T., Doupinik, J. R., Banks, P. M., Park, R. J. & Siren, J. C. *Radio Sci.* 12, 285 (1977).
- ⁸ Leadabrand, R. L., Baron, M. J., Petricks, J. & Bates, H. F. *Radio Sci.* 7, 747 (1972).
- ⁹ Watkins, B. J., Bates, H. F., Belon, A. E. & Hunsucker, R. D. *World Data Center-A Report UAG-28, Part II* 492 (1973).
- ¹⁰ Hunsucker, R. D. *Radio Sci.* 9, 335 (1974).
- ¹¹ Hunsucker, R. D. *World Data Center-A Report UAG-28, Part II* 490 (1973).
- ¹² Doupinik, J. R., Brekke, A. & Banks, P. M. *J. geophys. Res.* 82, 499 (1977).
- ¹³ Kan, J. R. & Heacock, R. R. *J. geophys. Res.* 81, 2371–2376 (1976).

Linear dichroism probes to study internal electric fields

LARGE electric fields outside a highly charged macro-ion or between any ion and its counter ion atmosphere form the basis of electrolyte theory. The fields manifest themselves in a number of macroscopic properties of such systems, but few, if any, successful measurements of such fields have been possible. We report here observations which suggest that optical probes can be used as field meters in anisotropic solvents. In aqueous solutions containing flow-aligned cylindrical micelles of cetyltrimethylammonium bromide (CTAB) several anisotropic anions exhibit linear dichroism effects which can be ascribed to orientation and perturbation of these chromophores by a radially directed field of about 10^8 V m^{-1} surrounding each $(\text{CTA}^+)_n$ cylinder.

Linear dichroism ($\text{LD} = A_{\parallel} - A_{\perp}$, definition and measuring techniques in refs 1–3) and absorption (A) spectra were recorded on several substances, added in small amounts (maximum 0.1%), in aqueous CTAB (20%) solution, in a Couette device at radial optical propagation. At the concentration and temperature used, this detergent forms practically infinite cylindrical micelles, with a diameter of $\sim 50 \text{ \AA}$, which are alignable in a flow gradient^{3,4}. The observation of a non-zero LD (Table 1) is not *a priori* unequivocal evidence for an interaction between the chromophore and the fields around the macro-ion, but one has first to correct for the so-called Wiener dichroism (= WLD, defined in ref. 5) which is the imaginary correspondence of form-birefringence. WLD has the shape of the absorption spectrum (WLD/A being constant), but its sign depends on the dielectric constant of the medium surrounding the chromophore. In micellar CTAB, WLD is positive for chromophores close to the cationic micelles and negative for peripherally located chromophores. Thus anions have positive, and cations negative WLD⁵. The observed LD was corrected for the expected WLD (Table 1). For ions like $\text{Cr}(\text{oxalate})_3^{3-}$ or other pseudo-cubic ions⁵, WLD is sufficient to explain the observed signals. This is also true for anisotropic cations, which by avoiding the macro-ion are in regions with comparatively weak electrostatic fields. With anisotropic anions the situation is different and after eliminating WLD, a sometimes complicated pattern of bands of any sign remains (Table 1, Fig. 1).

From the treatments of Labhart⁶, Liptay⁷ and Lin⁸ on molecules in electric fields, one has for the absorption difference $A_{\chi} - A$, due to an electric dipole allowed transition $b \leftarrow a$, at the frequency ω (A_{χ} is the absorption with an angle χ between the electric field and the electric vector of the polarised light; A is the absorption in the absence of field):

$$\text{ELD}\chi(\omega) = (A_{\chi} - A)\omega = \frac{\beta E^2}{10} A(\omega) S + \frac{\omega E^2}{10\hbar} \frac{\partial}{\partial \omega} \left\{ \frac{A(\omega)}{\omega} \right\} T + \frac{\omega E^2}{10\hbar^2} \frac{\partial^2}{\partial \omega^2} \left\{ \frac{A(\omega)}{\omega} \right\} U \quad (1)$$

$$\text{where } S = (3 \cos^2 \chi - 1) [(\hat{p}_{ab} \cdot \alpha(a) \hat{p}_{ab} - \frac{1}{3} T_r(\alpha(a))) + \beta((\hat{p}_{ab} \cdot p_{aa})^2 - \frac{1}{3} |p_{aa}|^2)]$$

$$T = [(2 - \cos^2 \chi) T_r(\Delta \alpha(b, a)) + (3 \cos^2 \chi - 1) \hat{p}_{ab} \cdot \Delta \alpha(b, a) \hat{p}_{ab} + 2\beta[(2 - \cos^2 \chi) p_{aa} \cdot \Delta p(b, a) + (3 \cos^2 \chi - 1) (\hat{p}_{ab} \cdot p_{aa}) \hat{p}_{ab} \cdot \Delta p(b, a)]]$$

$$U = [(2 - \cos^2 \chi) |\Delta p(b, a)|^2 + (3 \cos^2 \chi - 1) |\hat{p}_{ab} \cdot \Delta p(b, a)|^2]$$

where $\beta = 1/kT$, $\hat{p}_{ab}$ is a unit vector along the transition moment p_{ab} ; $\alpha(a)$ and $p_{aa} = \sum \epsilon r_i$ are the polarisability tensor and the permanent dipole moment, respectively, of the ground state. $\Delta \alpha(b, a)$ and $\Delta p(b, a)$ are the difference in polarisability and the difference in dipole moment, respectively, between the ground and the excited states. If dipole orientation effects⁹ predominate over

Table 1 Observed linear dichroism (LD) of various chromophoric ions in the aligned micellar CTAB(20%)/H₂O system

Chromophoric probe ion	Symmetry	Wavelength of extrema (nm) LD	<i>A</i>	LD <i>A</i>	WLD <i>A</i>	ELD* <i>A</i>	θ
<i>p</i> -Aminobenzenesulphonate ⁻	C _{2v}	297 251.5	295† 251.5	-0.042 +0.034	+0.02 +0.02	-0.06 +	$\pi/2$ §§ 0§§
Methyl orange (4'-dimethylaminoazobenzene-4-sulphonate ⁻)	C _{2v}	418	425	-0.029	+0.02	-0.05	0§§
Methyl red (4-dimethylaminobenzene-azobenzene-2'-carboxylate) ⁻	C _{2v}	515 410	512 410†	-0.012 +0.015	+0.020 +0.020	-0.03 +	0§ 0§
Benzoate ⁻	C _{2v}	276.5 268.5 262.0 255	276.8 269.8 263.8 256	+0.046‡ +0.040‡ +0.037‡ +	+0.02 +0.02 +0.02 +0.02	+0.03 +0.02 +0.02 +	$\pi/2$ § $\pi/2$ § $\pi/2$ § 0§
Co(III) (ethylenediaminetetraacetate) ⁻	C ₂	<245 553 380	538 380 610	-0.07 +0.012 -0.014	+0.02 +0.02 +0.02	-0.09 0 -0.03	0§ 0§ 0**
<i>trans</i> [Co(ethylenediamine) ₂ Cl ₂] ⁺	D _{2h}	610	610 460 <325	-0.014 -0.01 -0.014	-0.017 -0.017 -0.017	0 0 0	†† †† ††
Cr(oxalate) ₃ ³⁻	D ₃	570 415 270	570 417 270	+0.023 +0.029 +0.032	+0.020 +0.020 +0.027	0 0 0	†† †† ††
Co(ethylenediamine) ₂ CO ₃ ⁺	C ₂	520 358	525 360	-0.010 -0.010	-0.017 -0.017	0 0	†† **

Concentrations of added impurities were varied according to $A = 0.1 - 1.5$ (at $d = 0.1$ cm) without any noticeable variation in the LD/ A spectrum.

*Recorded LD adjusted for WLD estimated according to ref. 5.

†Shoulder.

‡Value referring to resolved component.

§Band polarisation confirmed in LD study in stretched polymer sheet.

¶d-d transition ${}^1T_{2g} \leftarrow {}^1A_{1g}$ in O_h^{12} .

**d-d transition ${}^1T_{2g} \leftarrow {}^1A_{1g}$ in O_h : isotropic but may in C_{2v} get a certain allowance along the C₂-axis, that is, parallel to $\hat{p}$

††Unpolarised isotropic transition.

‡‡No dipole moment.

§§Assignment according to ref. 11.

effects due to electronic perturbations (that is, T , U and $\alpha(a)$ can be neglected) we have

$$\frac{A_1 - A_2}{A} = \frac{A_{\theta=0} - A_{\theta=\pi/2}}{A} = \frac{1}{10} \left(\frac{p_{ab} \| E\|}{kT} \right)^2 (3 \cos^2 \theta - 1) \quad (2)$$

where θ is the angle between p_{ab} and p_{aa} . After scaling by $-1/2$ equation (2) applies to the geometry of the CTAB system ($\parallel$ = the direction of the micelle cylinders). In Table 1, θ values are given when p_{ab} is available from spectroscopic assignments and p_{aa} from geometrical considerations. For an ion the dipole moment is referred to the centre of mass as origin. A generally poor agreement indicates that the assumptions behind equation (2) are not justified here. But, we get an accurate order of magnitude of the field (or at least the minimum field) since the field appears as a squared term. Thus $(A_1 - A_2)/A = +0.03 \pm 0.01$ is consistent with $pE/kT = 0.39 \pm 0.06$ for a polar molecule with $\theta = 0$. If the permanent dipole moment is 5 D, which is reasonable for *p*-aminobenzenesulphonate at a neutral pH, we get $E = 2.5 \times 10^8$ V m⁻¹ ($\pm 20\%$).

A more satisfactory treatment should rest on a semi-empirical evaluation of S , T and U by electric dichroism spectroscopy. In Fig. 2 we show the $A_{\theta=\pi/2} - A$ spectrum of methyl red in the presence of an applied external electric field. As predicted by equation (1) the signal was found to be proportional to E^2 . The contribution from the T , 'polarisability', term is predominant and a curve with the shape of $\partial A/\partial \omega$ is obtained. Returning to the spectrum of methyl red at the surface of (CTA⁺)_n micelles (Fig. 1), we may now rationalise it, with the aid of the 'artificial' spectrum, in terms of an electrostatic field, radially directed from the (CTA⁺)_n, and with a strength of 1.45×10^8 V m⁻¹. A higher value results if the incomplete alignment of the micelles is taken into account.

The present findings also agree with the field expected from electrostatic theory. We assume that the observed phenomena take place for ions at a distance r from the charged surface of the macroion (r being less than the thickness of the ionic atmosphere). From

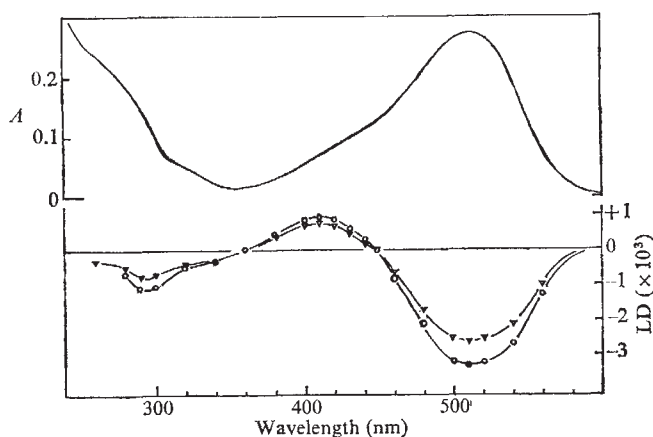
the difference in potential between bound and free counterions one may approximate the field as¹⁰

$$E = \frac{l \ln(R/r) \gamma e}{r d \epsilon} \quad (3)$$

where γ is the apparent degree of dissociation of counterions, R is the apparent radius of the macromolecule and r the radius of its free volume, d the average distance between neighbouring ionised groups and ϵ the dielectric constant of the solvent (water). In the present system we take $\gamma \ll 0.5$, $R/r = 0.2$ and $d = 5 \times 10^{-10}$ m, so in the region 5–10 Å from the cylinder we have fields ranging between 8×10^8 and 4×10^8 V m⁻¹.

Platt¹³ suggested the term electrochromism to designate all changes in optical absorption or emission of molecules in condensed phases due to external electric fields, thus including energy

Fig. 1 Linear dichroism LD = $A - A_0$ and absorption (A) of methyl red in 20% CTAB, 80% H₂O at a Couette flow of $G = 2,600$ s⁻¹ (▽) and 5,000 s⁻¹ (○). Optical path-length: 0.10 cm.



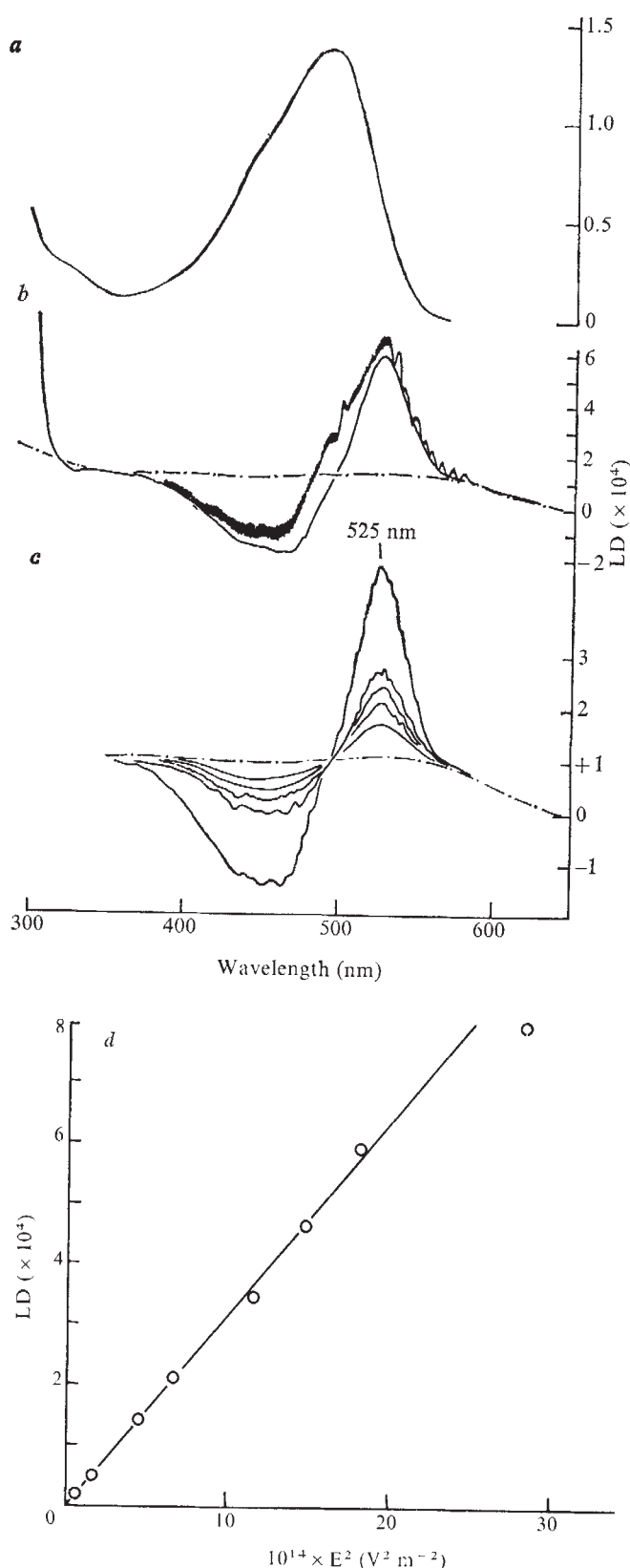


Fig. 2 *a*, Ψ Absorption and, *b*–*c*, electric linear dichroism (ELD = $A_{\parallel} - A_{\perp} = \pi/2 - A$) of methyl red in polystyrene sheet (thickness 0.0065 cm) obtained as the absorption difference with and without a longitudinal electric field (a field sinusoidally oscillating at 470 Hz between 0 and a given amplitude value, limited by flash-over around 4,000 V, was used together with lock-in electronics). *c*, Curves recorded immediately after applying the field (no orientation) at 0 (—), 1.6, 2.0, 2.3, 2.5 and 3.8×10^7 V m $^{-1}$ (certain orientation) at two spectral band widths: 2 nm (noisy curve) and 8 nm. Fringes due to interference in film, visible in the former spectrum. *d*, Steady state ELD signal at 525 nm, from the sample in *ac* plotted versus E^2 .

shifts as well as anisotropic phenomena (Kerr effect, electric dichroism). Much interest has recently centred around the internal fields in biomembranes¹⁴, solid polymers¹⁵ and so on, and the use of dye¹⁶ probes to study isotropic manifestations, such as shifts and intensity changes, of the solvent field has also been discussed. Here we report the possibility of discriminating the field effects by using anisotropic 'solvatochromism'.

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- ¹ Davidsson, Å. & Nördén, B. *Chem. Scr.* **9**, 49–53 (1976).
- ² Nördén, B. & Tjerneld, F. *Biophys. Chem.* **4**, 191–198 (1976).
- ³ Johansson, L. B.-Å., Lindblom, G. & Nördén, B. *Chem. phys. Lett.* **39**, 128–133 (1976).
- ⁴ Scheraga, H. A. & Backus, J. K. *J. Am. chem. Soc.* **73**, 5108–5112 (1951).
- ⁵ Nördén, B., Davidsson, Å. & Johansson, L. B.-Å. *Nature* **261**, 400–402 (1976).
- ⁶ Labhart, L. *Helv. Chim. Acta* **44**, 447–467 (1961).
- ⁷ Liptay, W. *Z. Naturforsch.* **20**, 272–289 (1965).
- ⁸ Lin, S. H. *J. chem. Phys.* **62**, 4500–4524 (1975).
- ⁹ Fredericq, E. & Houssier, C. *Electric Dichroism and Electric Birefringence* (Clarendon, New York, 1973).
- ¹⁰ Oosawa, F. *Polyelectrolytes* (Marcel Dekker, New York, 1971).
- ¹¹ Jaffe, H. H. & Orchin, M. *Theory and Applications of Ultraviolet Spectroscopy* (Wiley, New York, 1962).
- ¹² Douglas, B. E., Haines, R. A. & Brushmiller, J. G. *Inorg. Chem.* **3**, 1194–1198 (1963).
- ¹³ Platt, J. R. *J. chem. Phys.* **34**, 862–863 (1961).
- ¹⁴ Reich, R., Scheerer, R. & Sewe, K.-U. *Ber. Bunsenges. physik. Chem.* **80**, 245–246 (1976).
- ¹⁵ Hong, S. D. & Stein, R. S. *J. polym. Sci.* **13**, 1447–1459 (1975).
- ¹⁶ Kuhn, H. & Schweig, A. *Chem. phys. Lett.* **1**, 255–258 (1967).

Quasi-Fermi level measurement in an illuminated GaP photoelectrolysis cell

No experimental procedure is known for measuring directly the position of the minority carrier quasi-Fermi level, ϕ , at the surface of a semiconductor electrode¹. ϕ is an important parameter in studies of photoelectrolysis (p.e.) (ref. 2), of surface states, of catalysis, of the anomalous intensity dependence of the photovoltage of Schottky barriers³, and of the efficiency and stability against corrosion of low-cost photoelectrochemical solar cells. Hence a means for determining ϕ should be of wide interest. I describe here a technique which results from an effort to measure changes in the potential versus standard calomel electrode (SCE), V_{Au} , of a thin porous Type B gold film⁴ evaporated on the surface of n-GaP in a $\langle Au/GaP/K_2SO_4/Pt \rangle$ p.e. cell when the cell is illuminated with photons having energy $h\nu \geq E_G$, the 2.23 eV energy gap of GaP. Changes in V_{Au} of $\sim +1.0$ V have been observed. It is shown below that $V_{Au} \sim \phi$, the minority hole quasi-Fermi level in the illuminated n-GaP anode. This series of measurements provides other valuable information. The valence band (VB) of the Au/GaP anode is shown to > 0.21 V below, or anodic to, the OH^-/H_2O redox potential, so that oxidation of OH^- is possible. But, p.e. with liberation of H_2 and O_2 is shown to be highly improbable. The results provide evidence that the Type B gold film does not protect the GaP surface against electrochemical corrosion. The variation with time of V_{Au} provides a sensitive indicator of the existence and rate of GaP corrosion. The technique for determining ϕ should be applicable to semiconductors, both n- and p-type, other than GaP.

The single crystal n-GaP (Metals Research) anode was prepared with a sintered Au + 12%Ge alloy ohmic contact on the rear surface and the front surface was etched in aqua regia. A ring of electrically insulating Sealit (Fisher Scientific) 1 mm wide was painted on the front surface at its perimeter, leaving a clear area in the centre. A 16 nm thick Au film was rapidly evaporated (3 s) to cover both the Sealit ring and the clear area. Electrical

contact to the Au film was by means of a Au-foil ring placed between the gold-covered Sealit and the polyethylene cell wall, which had a 0.6-cm diameter hole through which illumination reached the 0.12-cm² exposed Au surface.

A schematic view of the cell and electrical connections is given in Fig. 1. Illumination intensity L approximates 2 am1 suns ≈ 150 mW cm⁻². Potentials were measured between the Au ring and SCE (V_{Au}), between the GaP ohmic contact and SCE (V_{GaP}), and between the GaP ohmic contact and the Pt cathode (V_{cell}). V_{Au} , V_{GaP} and V_{cell} were first noted for the open-circuited (o.c.) cell, dark and then illuminated. Measurements of these potentials were then repeated for the short-circuited (s.c.) cell, along with observations of current I and time T . No external potential was applied at any time to the cell. N₂ was bubbled through the electrolyte during the measurements.

For the o.c. dark cell, $V_{Au} \sim V_{GaP} \sim +0.09$ V (SCE); for the o.c. illuminated cell $V_{Au} = -0.03$ V, $V_{GaP} = -1.0$ V (initially). We interpret this illuminated result to mean that the initial energy difference between the electron Fermi level, $E_a^i = V_{GaP}$, and $\phi_p = V_{Au}$, equals 0.97 V. The result also shows that illumination has caused a -0.13 V cathodic shift in the Fermi level of the Au film. The photovoltage generated through separation of electron-hole pairs by the electric field of the surface Schottky barrier causes V_{GaP} to shift to -1.0 V versus SCE.

The s.c. results, more characteristic of normal cell operation, are given in Fig. 2. The outstanding feature of the data is the immediate rise of V_{Au} from $+0.08$ V to $+0.92$ V (SCE) when the Au/GaP anode is illuminated. The OH⁻/H₂O redox potential is at $+0.71$ V (SCE) for the pH 4.6 electrolyte so that oxidation of OH⁻ ions by the photoholes is initially possible. There is a sustained rise of I during the first few minutes of illumination together with the associated parallel shift in the cathodic direction of V_{Au} and V_{GaP} . Current I continues to rise even when V_{Au} becomes cathodic to the OH⁻/H₂O potential, indicating that the major part of I is due to oxidation of some species other than OH⁻. There is an initial difference of 0.99 V for $V_{Au} - V_{GaP}$, equal to the initial o.c. potential difference, and the reduction of this difference with time as V_{Au} falls off more rapidly with time than does V_{GaP} .

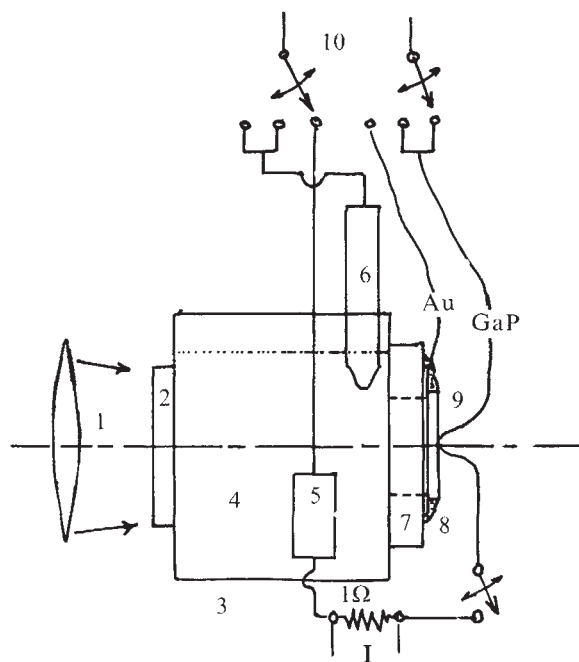


Fig. 1 Schematic view of cell for measuring V_{Au} . 1, Light from 150-W quartz-I₂ lamp; 2, quartz cell window; 3, polyethylene cell, 2.5 cm wide; 4, 0.1 M K₂SO₄ at pH 4.6; 5, large Pt black cathode; 6, Luggins capillary and SCE; 7, polyethylene mount for anode; 8, Sealit cement; 9, Au/n-GaP anode; 10, DMM having $R_{in} > 10^{10}$ Ω .

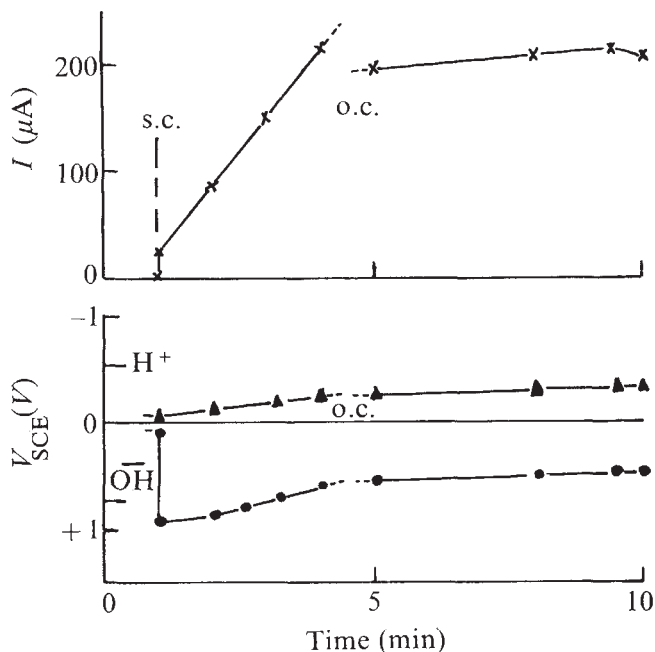


Fig. 2 Time variation of gold-film potential (●), bulk n-GaP potential (▲) and cell current (×) during the first 10 min of s.c.-illuminated operation. Note the gold-film potential is anodic to the OH⁻/H₂O redox potential for the first 2 min of operation so that oxidation of OH⁻ by the photoholes is possible.

Agitation of the electrolyte at $T = 14$ min seems to have ruptured the Au film; V_{Au} immediately fell to $+0.08$ V and the current to 37 μ A. Visual inspection of the Au surface from outside the cell showed several dark patches on the Au/GaP surface. Inspection later of the cleaned GaP surface revealed deep corrosion craters directly below where the dark patches had been noted earlier.

Tests of other gold-covered anodes revealed a pattern of behaviour similar to the above, with the exception that the gold surface films seemed to be intact.

Before the results are discussed, the concept of the quasi-Fermi level will be introduced and defined, followed by a brief examination of key features of the Type B film hypothesis⁴.

Because of the low rate of charge transfer between CB and VB of a semiconductor, non-equilibrium concentrations of electrons, $n_0 + \Delta n$, in the CB, and of minority carrier holes, $p_0 + \Delta p$, in the VB, are generated by illumination absorbed in the surface space charge region. Each distribution has in general a different mean free energy and a corresponding quasi-Fermi level. Unless L is very large, $\Delta n < n_0 = 2 \times 10^{17}$ cm⁻³, so that the electron mean free energy and Fermi level remain virtually unchanged by the illumination. But $\Delta p \gg p_0$, the minority hole concentration in the n-GaP, so that Φ_p can be changed drastically by the illumination. Thus

$$\Phi_p = A + kT/e \{ \ln [(p_0 + \Delta p)/n_i] \} \quad (1)$$

$$\Delta p \sim L \tau_p \quad (2)$$

where n_i is carrier concentration and A the electron Fermi level in an intrinsic GaP semiconductor. τ_p is the hole lifetime near the n-GaP surface.

The model⁴ for the Type B film assumes the existence of a 2 nm thick semi-insulating oxide film between the Au film and the GaP substrate. Photoholes from the n-GaP can tunnel through the oxide film into the Au film. Positive charge and potential build up in the Au film until the rate at which holes

enter the film equals the rate at which holes oxidise redox species, such as OH^- , in the electrolyte. To maintain the net flow of holes and the current in the s.c. cell, Φ_p will be more anodic than V_{Au} , and V_{Au} more anodic than the redox potential of the oxidised species. If the oxide is thin and easily tunnelled through, indicated by an abrupt rise of V_{Au} when L is turned on, one expects $V_{\text{Au}} \sim \Phi_p$. Also in this case the maximum value of $V_{\text{Au}} - V_{\text{GaP}}$ for the o.c. and s.c. cells should be almost equal, in agreement with the results above.

For the s.c. results it is possible to demonstrate a set of relationships between the cathodic shifts of V_{Au} and V_{GaP} and the sustained rise of I during the first few minutes of cell operation. (1) The cathodic shift of V_{GaP} is equal to the shift of the Pt cathode potential as increasing amounts of H are reduced and absorbed on the Pt surface by the photoelectron current originating in the CB of the n-GaP. (2) For constant L , equation (1) indicates that $V_{\text{Au}} - V_{\text{GaP}}$ should remain constant with time. This is not observed, however, instead V_{Au} shifts more rapidly in the cathodic direction than does V_{GaP} . (3) We attribute this observed difference in rate to electrochemical corrosion of the GaP surface beneath the Au film, according to the following sequence: GaP corrosion $\rightarrow$ increased surface recombination rate for electrons and holes $\rightarrow$ reduction in $\tau_p \rightarrow$ reduction in $\Delta p \rightarrow$ reduction in $\Phi_p > V_{\text{Au}} - V_{\text{GaP}}$ decreases with increasing time. (4) The sustained rise in I during the first 3 min may be due to a corrosion-generated local increase of H^+ under the Au film⁵ which in turn increases the corrosion rate and I . The intensity L establishes an upper limit for I so that I peaks and then begins to fall off as corrosion reduces τ_p and Δp .

If the above analysis is valid, then the observation of a reduction in $V_{\text{Au}} - V_{\text{GaP}}$ with time can be used as a tool for determining the existence and rate of electrochemical corrosion under the protective Au film. It could thus serve to evaluate photoelectrochemical solar cells and their rate of corrosion in various electrolytes.

Data of Fig. 2 also provide information about the flat-band potential, ϵ_{fb} , of the Au/GaP anode and the possibility of p.e. The VB edge of the Au/GaP anode is more than 0.21 eV below the $\text{OH}^-/\text{H}_2\text{O}$ redox potential since Φ_p must be above the VB edge; Δp in equation (1) cannot exceed the density of hole states in the VB, ϵ_{fb} is approximately 2.23 eV above the VB edge.

During the first few minutes of s.c. operation, when V_{Au} is anodic to the $\text{OH}^-/\text{H}_2\text{O}$ potential, oxidation of OH^- by holes in the Au film is possible. In the absence of an external potential p.e. is not possible since $V_{\text{Au}} - V_{\text{GaP}} \approx \Phi_p - E_i^n < 1.23$ V, the p.e. threshold, at all times. Equation (1) shows that a 10^4 -fold increase in L and Δp would be required to make $V_{\text{Au}} - V_{\text{GaP}} \approx 1.23$ V. A Pd film evaporated on to the surface of the Au film⁴ will not alter this conclusion (in agreement with some preliminary results).

Finally, we may conclude that in almost neutral aqueous electrolytes a n-GaP surface cannot be protected from electrochemical corrosion through use of a porous gold film evaporated on to its surface.

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¹ Gerischer, H. *Proc. Int. Conf. Photochem. Conversion Storage sol. Energy Univ.* Western Ontario, London, Ontario, Canada (August, 1976).

² Manassen, J., Cahen, D., Hodes, G. & Sofer, A. *Nature* **263**, 97–100 (1976).

³ Gerischer, H. *J. electroanal. Chem.* **58**, 263–274 (1975).

⁴ Nakato, Y., Abe, K. & Tsubomura, H. *Ber. Bunsenges. physik. Chem.* **80**, 1002–1007 (1976).

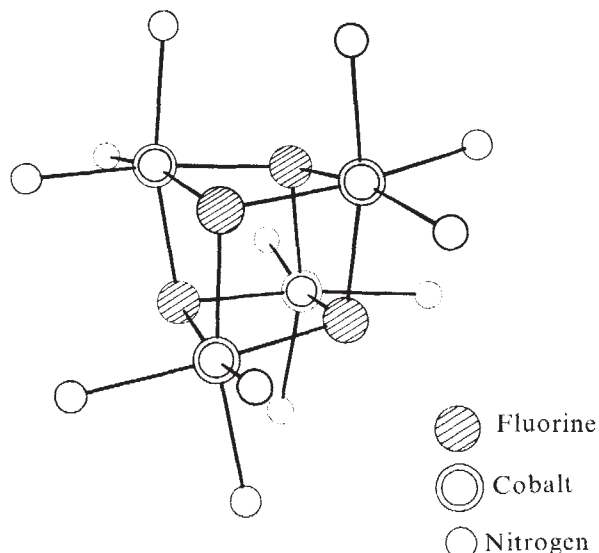
⁵ Gerstner, M. E., Harris, L. A. & Wilson, R. H. Abst. 442 RNP, Philadelphia Meeting Electrochem. Soc. (May, 1977).

The first cubane-type fluoro-bridged cluster

CUBANE-TYPE clusters containing the unit M_4A_4 have been the subject of many investigations. Several of these systems have been characterised by X-ray structure determination. Such clusters are important from a bioinorganic point of view, for example, Fe_4S_4 clusters in iron-sulphur proteins¹. From a catalytic point of view, clustered compounds are becoming increasingly important as models for heterogeneous catalysts, and even as catalysts by themselves². Monoatomic ligands (for example, A=S, Cl, P, Br, Se) have been reported as bridging ligands in cubane-type clusters, but larger groups are known in which C, N, and O act in a similar way³. No cubane-type clusters have been previously reported with the highly electronegative F^- ion as a bridging ligand. We describe here the synthesis and structure of the first fluoro-bridged cubane-type cluster, having the formula $[\text{M}_4\text{F}_4(\text{L})_{12}] (\text{BF}_4)_4$, in which $\text{M}=\text{Mn}$, Cd , Co and $\text{L}=\text{N-ethylimidazole}$ or N-propylimidazole . On reaction of $\text{M}(\text{H}_2\text{O})_6 (\text{BF}_4)_2$ in ethanol-triethyl orthoformate with an ethanolic solution of the ligand, finely-divided white powders (Mn, Cd) or purple crystals (Co) separate, analysing as $\text{M}(\text{ligand})_4\text{BF}_5$. In these circumstances the F^- ions are spontaneously generated by a slow decomposition of the BF_4^- ion. Such a decomposition of tetrafluoroborates, yielding fluoro complexes, has been reported in a few other cases, yielding polymeric fluoro-bridged systems⁴, dimers (M_2F_2)⁵ and also monomers MF_2 (ligand)₄ (ref. 6).

X-ray powder diagrams showed all compounds to be mutually isomorphous. Infrared spectra indicate the presence of uncoordinated BF_4^- ions. Ligand-field spectra of the Co(II) compounds indicate a six-coordinate octahedrally-based geometry for Co(II) , whereas magnetic measurements at low temperature (2–80 K) show the presence of a weak anti-ferromagnetic exchange ($-J \sim 1 \text{ cm}^{-1}$) between the Co(II) ions. The strong far-infrared absorptions around 300 cm^{-1} suggest the presence of bridging M-F-M units. From these physical measurements a cubane-type structure was proposed. To verify this

Fig. 1 Cubane-type cluster of $\text{Co}_4\text{F}_4 (\text{N-ethyl imidazole})_{12}^{4+}$ with the first coordination sphere around cobalt. Mean distances and angles are: $\text{Co-F} = 2.14(2) \text{ \AA}$; $\text{Co-N} = 2.19(3)$; $\text{Co-Co} = 3.29(2)$; $\text{F-F} = 2.71(1)$; $\angle \text{F-Co-F} = 78.5(5)^\circ$; $\angle \text{Co-F-Co} = 100.5(5)^\circ$.



proposal a crystal-structure determination was undertaken.

An octahedrally shaped crystal of $\text{Co}(\text{N-ethylimidazole})_2\text{BF}_4$ having the approximate edges of 0.2 mm was selected. The crystal belongs to the cubic space group $\text{Pa}\bar{3}$ with $a = 25.797(1) \text{ \AA}$. The experimental density of about 1.4 g cm^{-3} indicates that 32 CoL_3BF_4 units are present in the unit cell. One unit occupies an eightfold position (the threefold axis); the other one is in a general 24-fold position. The cluster, consisting of four CoL_3BF_4 units, has trigonal symmetry. At the present stage of refinement $R_F = 0.13$. The cluster is drawn in Fig. 1. The trigonal clusters can be considered as being built up by two interpenetrating tetrahedrons. The F_4 tetrahedron is symmetric; all F-F distances are equal within experimental error. The Co_4 tetrahedron has a larger edge and is slightly elongated in the trigonal direction: $\text{Co-Co} = 3.28\text{--}3.31 \text{ \AA}$. This relatively large Co-Co distance suggests at best a weak Co-Co bonding. The Co-F and Co-N distances have normal values compared to other octahedrally coordinated Co(II) compounds. The BF_4^- ions are located outside the cluster; one BF_4^- is on the trigonal axis, whereas the other anion is in a general position. Due to disorder the orientation of the BF_4^- anions cannot be determined accurately.

The Co-F-Co angles of about 101° are important in understanding the low-temperature magnetic behaviour of this compound. According to De Jongh and Block⁹ bond angles larger than 90° should result in antiferromagnetic behaviour, in agreement with the experiments.

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- ¹ Holm, R. H. *Endeavour* **34**, 38-45 (1975).
- ² Demitras, G. C. & Muetterties, E. L. *J. Am. chem. Soc.* **99**, 2796-7 (1977).
- ³ Bertrand, J. A. & Eller, P. G. *Progr. inorg. Chem.* **21**, 29-53 (1976).
- ⁴ Guichelaar, M. A., van Hest, J. A. M. & Reedijk, J. *Inorg. nucl. Chem. Lett.* **10**, 999-1004 (1974).
- ⁵ Jansen, J. C. & van Koningsveld, H. *Cryst. Struct. Commun.* **5**, 441-445 (1976).
- ⁶ Smit, S. & Groeneveld, W. L. *Inorg. nucl. Chem. Lett.* **11**, 277-80 (1975).
- ⁷ Babel, D. Z. *Anorg. Allg. Chem.* **369**, 117-30 (1969).
- ⁸ Clarke, P. J. & Milledge, M. J. *Acta Crystallogr.* **B31**, 1543-53 (1975).
- ⁹ de Jongh, L. J. & Block, R. *Physica* **79B**, 568-93 (1975).

Hydrothermal manganese in the Galapagos Rift

HYDROTHERMAL emanations originating at mid-ocean ridges have been thought¹⁻⁵ to provide a substantial source of manganese to the ocean but the evidence supporting this hypothesis has been indirect. Anomalous manganese concentrations have been measured in naturally occurring systems where seawater is in direct contact with lava flows⁶⁻⁸. Laboratory studies have shown that seawater tends to leach manganese from basalts at elevated temperatures and pressures⁹⁻¹¹. Anomalous high manganese accumulation rates have also been determined for sediments adjacent to active ridge systems, most notably the East Pacific Rise¹²⁻¹⁴. No measurements of manganese concentrations in seawater near mid-ocean ridges, or in hydrothermal fluids emanating from these ridges, have yet been made, however. We report here the results of the first such direct measurements, which show that manganese is being injected into the deep sea by hydrothermal circulation of seawater

through newly-formed oceanic crust.

We analysed water samples collected in the Galapagos Rift during two cruises of the RV Melville (Pleiades expedition, Legs 1 and 2). These samples were collected from hydrocasts and with a remote-controlled sampler attached to the Deep Tow vehicle of the Scripps Marine Physical Laboratory¹⁵. Unfiltered aliquots of these samples were acidified to pH 2 with redistilled 6M HCl. Manganese was determined by addition of radioactive ⁵⁴Mn,

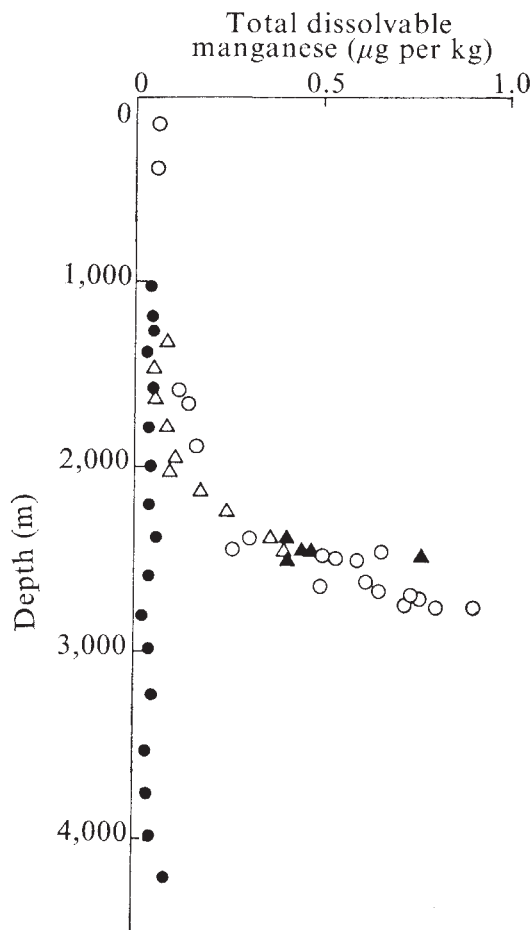


Fig. 1 The concentrations of TDM plotted against depth for samples collected from hydrocasts at two Pacific locations. ●, Samples collected during the Geosecs program at station 343 ($16^\circ 31.6' \text{N}$, $123^\circ 1.4' \text{W}$). △ And ○, samples from eight Pleiades Expedition stations taken at the Galapagos Rift survey site ($0^\circ 35.5'$ to $0^\circ 48.0' \text{N}$, $86^\circ 4.7'$ to $86^\circ 10.2' \text{W}$; see map ref. 15) △, Pleiades Leg 1; ○, Pleiades Leg 2. ▲, Deep Tow samples, although results for samples 7-0 and 8-3 are not plotted. The size of the symbols approximates the analytical uncertainty of the measurements.

adjustment of the pH to 8, extraction of manganese into 8-hydroxyquinoline in chloroform, and back extraction into 3M HNO_3 (ref. 1). Manganese concentration in the 3M HNO_3 solution was measured using an atomic absorption spectrophotometer with a graphite furnace and the yield was determined by counting the 835 keV ⁵⁴Mn γ ray. From these data and the volumes of the initial sample and concentrate, the manganese concentration of the initial sample was calculated. This value, which is believed to include all dissolved and particulate manganese except that fraction in the lattice of silicate minerals, is called 'total dissolvable manganese' (TDM)¹.

Figure 1 shows how TDM concentrations vary with depth at two separate locations. The shaded circles represent samples collected during the Pacific Geosecs program at station 343 ($16^\circ 31.6' \text{N}$, $123^\circ 1.4' \text{W}$). Of the locations at which we have made these measurements, station 343 is the closest geographi-

cally to the Galapagos Rift. Below 1,000 m, the TDM profile at this station is typical, with concentrations ranging from 0.017 to 0.087 μg per kg. Also shown in Fig. 1 are the TDM concentrations found for the samples collected from hydrocasts over the Galapagos Rift. They include samples taken at one station during Leg 1 and seven stations during Leg 2. These samples show TDM concentrations ranging from 0.046 to 0.90 μg per kg, with a maximum at the depth of the ridge crest in the study area. We believe that these anomalously high concentrations are the result of the injection of hydrothermal plumes into this area. Furthermore, the fact that the TDM concentration increases rapidly with increasing depth implies scavenging of hydrothermal manganese from the water column.

In addition to the high manganese concentrations found in the hydrographic profiles, we have made direct measurements of manganese in hydrothermal plume samples collected in the Galapagos Rift by the Deep Tow sampling system. The hydrothermal origin of these plume samples has been unequivocally demonstrated by measurements of excess ^3He and ^{222}Rn (ref. 16) as well as by the hydrographic evidence¹⁵. The TDM results for the Deep Tow samples are listed in Table 1, together with the ^{222}Rn results¹⁶ for the same samples. Sample 8-3, which had a ^{222}Rn anomaly of 80 d.p.m. per 100 kg, had a small positive $\delta^3\text{He}$ anomaly, and sample 7-0, which had a ^{222}Rn anomaly of 191 d.p.m. per 100 kg, had a large $\delta^3\text{He}$ anomaly. These data show that there is a one-to-one correspondence between the presence of very high TDM concentrations, the hydrographic identification of plumes, and the presence of excess quantities of helium and radon. The TDM concentration in plume sample 7-0 is 50 times the concentration in the surrounding bottom water. TDM is thus an exceptionally sensitive tracer of hydrothermal fluids: dilutions of the order of one part in 10^6 parts bottom water should be measurable.

Table 1 Total dissolvable manganese measurements on samples collected with the Deep Tow vehicle on the Galapagos Rift (0°48'N, 85°55'W), Pleiades Expedition Leg 1

Tow no.-bottle no.	TDM (μg per kg)	Excess ^{222}Rn ¹⁵ (d.p.m. per 100 kg)
Samples with potential temperature spikes		
7-0	24	191
8-3	2.1	80
Near-bottom samples with no temperature spikes		
7-1	0.41	39
7-2	0.41	49
8-1	0.77	—
8-5	0.48	—
8-7	0.48	—

Sample 7-0 was collected from a hydrothermal plume with a potential temperature anomaly of +0.20 °C relative to the surrounding bottom water¹⁵. Assuming that hydrothermal water in contact with basalt has a temperature of 100 °C (ref. 17), and that a negligible fraction of the TDM in sample 7-0 was scavenged before sampling, we calculate that the manganese concentration in the undiluted hydrothermal solution is 12 mg per kg. This value agrees well with predictions based on measurements of dissolved manganese in seawater basalt systems⁶⁻¹¹.

Our measurements provide the first direct evidence that manganese is injected into the deep water column as a result of leaching from newly-formed crust by hydrothermally circulating seawater. Although further measurements are required to establish the areal and temporal variability of this process, our data suggest that hydrothermal sources represent an important part of the manganese cycle in seawater.

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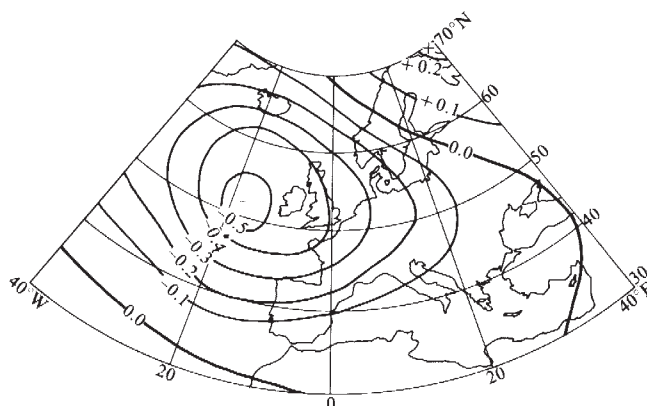
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- 1 Bender, M., Klinkhammer, G. & Spencer, D. *Deep-Sea Res.* (in the press).
- 2 Seyfried, W. & Bischoff, J. J. *Earth planet Sci. Lett.* **34**, 71-77 (1977).
- 3 Elderfield, H. *Mar. Chem.* **4**, 103-132 (1976).
- 4 Lyle, M. *Geology* **4**, 733-736 (1976).
- 5 Corliss, J. B. *J. geophys. Res.* **76**, 8128-8138 (1971).
- 6 Elderfield, H. *Mar. Geol.* **13**, M1-M6 (1972).
- 7 Ferguson, J. & Lambert, I. B. *Econ. Geol.* **67**, 25-37 (1972).
- 8 Oallison, J. *Nature* **255**, 138-141 (1975).
- 9 Bischoff, J. L. & Dickson, F. W. *Earth planet. Sci. Lett.* **25**, 385-397 (1975).
- 10 Mott, M. J., Corr, R. F. & Holland, H. D. *Geol. Soc. Am. Abs. Program.* **6**, 879 (1974).
- 11 Hajash, A. *Contr. Miner. Petrol.* **53**, 205-226 (1975).
- 12 Bostrom, K. & Peterson, M. N. *Econ. Geol.* **61**, 1258-1265 (1966).
- 13 Bender, M. *et al. Earth planet Sci. Lett.* **12**, 425-433 (1971).
- 14 Dymond, J. & Veeh, H. H. *Earth planet Sci. Lett.* **28**, 13-22 (1975).
- 15 Weiss, R. F., Lonsdale, P. F., Lupton, J. E., Bainbridge, A. E. & Craig, H. *Nature* **267**, 600-603 (1977).
- 16 Lupton, J. E., Weiss, R. F. & Craig, H. *Nature* **267**, 603-604 (1977).
- 17 Ross, D. A. *Science* **175**, 1455-1457 (1972).

Solar influence on North Atlantic mean sea level pressure

A PRINCIPAL component analysis (PCA) of mean sea level (MSL) pressure data for the North Atlantic-European sector of the Northern Hemisphere has revealed evidence of a solar-terrestrial relationship. The principal components represent independent atmospheric circulation anomaly patterns and the coefficients give the variation in time of the contribution of each anomaly pattern^{1,2}. The data set described here comprises 101 winter MSL pressure anomalies (relative to the 1874 to 1974 winter average) on a 25-point, 20° longitude by 10° latitude grid covering the area 40°W-40°E, 30°N-70°N. Winters (December-February) are dated by the year of the January. The suitability of this grid for calibrating proxy data for the European sector is being investigated as a preliminary stage to the derivation of Holocene climatic information³. The data were obtained from the UK Meteorological Office,

Fig. 1 Second principal component of winter MSL pressure anomalies (mbar). Based on data for the period 1874-1974.



and are likely to contain inhomogeneities due to different analysis techniques⁴ and so on. Some errors have become apparent in the course of the analysis and in general are confined to negligible low variance principal components. Missing data were replaced by the 101-yr mean for the grid point. Similar principal components were obtained by repeating the analysis for 30-yr sub-intervals. A slight latitudinal shift in the patterns occurred and there was some change in the relative importance of the principal components. Detailed results of the PCA are to be published elsewhere.

The second principal component (PC2) accounts for 20% of the variance of the original data set. The pattern is dominated, when its coefficient is positive, by a large negative anomaly area to the south of Iceland and west of the UK (Fig. 1). Positive anomalies occur over north-east Scandinavia. A positive contribution of PC2, therefore, represents intensified cyclonic development centred around 50°N 20°W, corresponding to a southward displacement

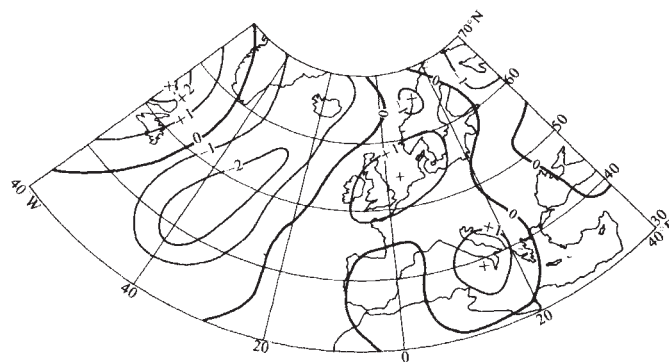


Fig. 3 500 mbar height change (gpdm) in the 24 h following a solar flare (mean of 81 events). (After Schuurmans⁶.)

high solar activity lends added support to the reality of the solar-terrestrial relationship. As solar flare activity is enhanced at sunspot maximum, the longer period solar effect on the atmospheric circulation seen in PC2 arises through the modulation throughout the 11-yr sunspot cycle of the short-term solar flare effects on the circulation. The physical mechanism by which low energy solar particles emitted in flare events can affect the atmospheric circulation has yet to be identified. The pattern of PC2 is very similar to the MSL pressure difference between sunspot maximum and minimum presented by Parker⁸, based on January data since 1750. An 11-yr cycle is also present in the S index, a measure of southerliness over the UK⁹. This variation in southerliness over the eastern Atlantic is a common feature of all the MSL pressure patterns associated with solar variation discussed here.

The coefficients of PC2, passed through a low pass binomial filter designed to reduce the amplitude of wavelengths less than 10 yr by 50% or more⁵, are shown in Fig. 4. The dates of sunspot maxima are arrowed¹⁰. An in-phase relationship is present during the 1880s and throughout the period 1920–60. It breaks down, however, between 1890 and 1920 and possibly during the 1960s. The relationship between North Atlantic sea temperature and sunspot num-

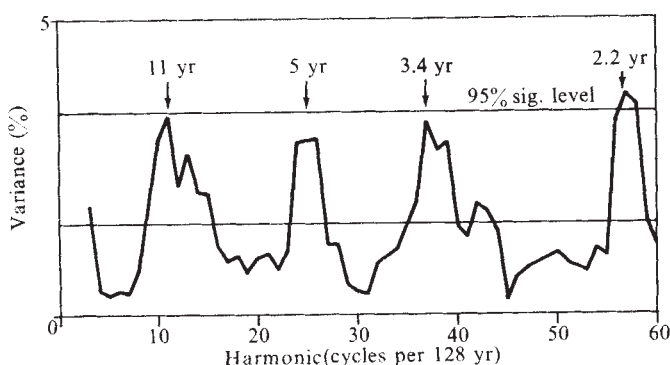


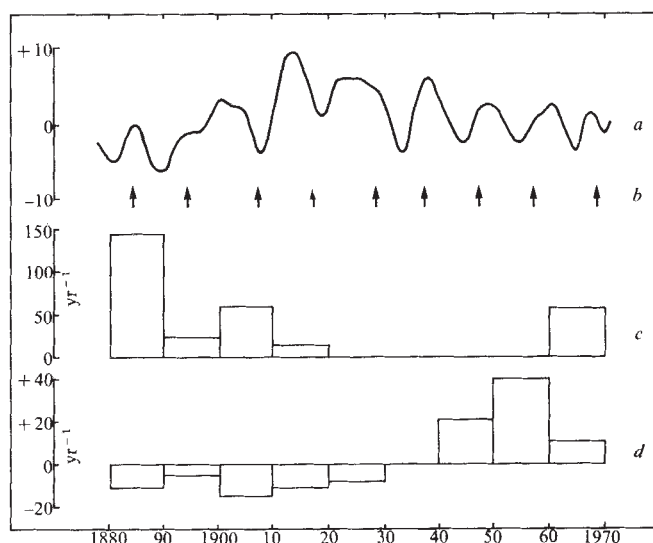
Fig. 2 Variance spectrum of the coefficients of PC2.

of the Iceland Low and enhanced southerly airflow over the North Sea. The pattern is inverted if its coefficient is negative.

The variance spectrum of the time series of the coefficients of the second principal component is shown in Fig. 2. Spectral peaks occur centred at 11, 5.0, 3.4 and 2.2 yr. The 11 and 2.2-yr peaks touch and surpass respectively the 95% significance level for an *a priori* choice of period, assuming a white noise null continuum⁵. The 2.2-yr peak may be associated with the quasi-biennial oscillation, but it is close to the Nyquist frequency. The other three peaks I tentatively assign to the 11-yr sunspot cycle and its harmonics; they are jointly significant at the 95% level⁵. A conservative estimate of the area enclosed by these three peaks is that 25% of the time variance of PC2 is associated with these cycles. This represents 5% of the total variance in the original data set.

Further evidence that the 11-yr cycle in the coefficients of PC2 is associated with solar variation is found by comparing the spatial pattern of PC2 with the pattern of 24-h 500-mbar height change following samples of individual solar flares⁶. Schuurmans' map (Fig. 3) shows an area of negative 500-mbar height anomalies (which can be interpreted as an anomalous upper air trough) over the mid North Atlantic and positive anomalies (an anomalous upper air ridge) over western Europe, with enhanced upper air southerlies over the eastern North Atlantic. The area of negative MSL pressure anomalies in PC2 is situated beneath the region of upper air divergence just ahead of the anomalous upper air trough. Such a region is favourable for cyclonic development and intensification⁷. This close correspondence between PC2 and the upper air circulation anomaly patterns associated with

Fig. 4 a, Coefficients of PC2, smoothed with a 9 weight low pass binomial filter⁵. b, Dates of sunspot maxima¹⁰. c, Volcanic dust veil index, 10-yr averages¹⁴ updated to 1969 (H. H. Lamb, personal communication). d, Relative sunspot numbers, 10-yr average anomalies relative to 1949–71 mean¹⁰.



hers, discussed by Muir¹¹ and Colebrook^{12,13} is also weakest about the turn of the century. This apparent variability in the strength of the North Atlantic solar-terrestrial relationship requires further investigation before its predictive potential can be realised. There are three possible explanations for the long-term variability. First, sunspot numbers may not be a good indicator of the particular component of the solar output affecting the atmospheric circulation. Second, the volcanic dust loading of the stratosphere was minimal during the period 1920–1960¹⁴ (Fig. 4c) and this may have resulted in a stronger solar control on the atmospheric circulation during that period. At other times, the volcanic control may mask the solar effects. Third, the amplitude of the sunspot cycle was far greater after 1920 than before (Fig. 4d).

The principal component analysis of the MSL pressure data is to be extended to other sectors of the Northern Hemisphere. Proxy data for areas where the solar-terrestrial relationship is strongest are to be used to lengthen the record of this 11-yr climatic variation and to identify the cause of the longer-term variation in its strength.

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- ¹ Kutzbach, J. E. *J. appl. Met.* **6**, 791–802 (1967).
- ² Craddock, J. & Flood, C. Q. *Jl R. met. Soc.* **95**, 576–593 (1969).
- ³ Kelly, P. M. *Palaeogeog. Palaeoclim. Palaeoecol.* (in the press).
- ⁴ Williams, J. & van Loon, H. *Mon. Weath. Rev.* **104**, 1354–1361 (1976).
- ⁵ W.M.O. T.N. No. 79 *Climatic Change* (W.M.O., Geneva, 1966).
- ⁶ Schuurmans, C. J. E. *Meded. Verh. K. ned. met. Inst.* **92** (1969).
- ⁷ Sutcliffe, R. C. & Forsdyke, A. G. Q. *Jl R. met. Soc.* **76**, 189–217 (1950).
- ⁸ Parker, B. N. *Met. Mag.* **105**, 33–44 (1976).
- ⁹ Kelly, P. M. *Phys. Bull.* **27**, 305–307 (1976).
- ¹⁰ Lamb, H. H. *Climate: Present, Past and Future 1* (Methuen, London, 1972).
- ¹¹ Muir, M. S. *Nature* **266**, 475–476 (1977).
- ¹² Colebrook, J. M. *Nature* **263**, 576–577 (1976).
- ¹³ Colebrook, J. M. *Nature* **266**, 476 (1977).
- ¹⁴ Lamb, H. H. *Phil. Trans. R. Soc.* **226**, 425–533 (1970).

Copper exclusion as a mechanism of heavy metal tolerance in a green alga

ORGANISMS isolated from environments polluted by heavy metals are often tolerant of those metals^{1–5}. Tolerance is usually accompanied by metal uptake equal to or greater than that of non-tolerant organisms^{2,6–10}; the accumulated metals seem to be chemically detoxified and/or physically sequestered to render them inactive^{2,6,7,11–13}. Because heavy metal tolerant algae¹⁰, plants², yeast⁷ and invertebrates^{8,10} have been found not to restrict metal uptake, metal exclusion has been considered a rare mechanism of tolerance⁶. I report here, however, that a copper-tolerant strain of the common unicellular green alga *Chlorella vulgaris* excludes copper.

Two strains of *Chlorella vulgaris* Beijerinck 1890 var. *vulgaris* Fott et Nováková were isolated from the River Hayle, which drains disused copper mines in Cornwall, England. The non-tolerant strain came from a site upstream of mining influence where copper was not detectable in the water (≤ 0.002 mg l⁻¹). The tolerant strain came from a polluted site with a total copper concentration in the water of 0.12 ± 0.03 mg l⁻¹ (mean $\pm$ s.e.m. of four samples taken during 1975–76.) To eliminate any non-genetic variation between the isolates, clones of each strain were subcultured approximately 30 times on Bold's basal medium agar¹⁴.

The effect of copper on the growth rates of the tolerant and non-tolerant strains is shown in Fig. 1. Although copper

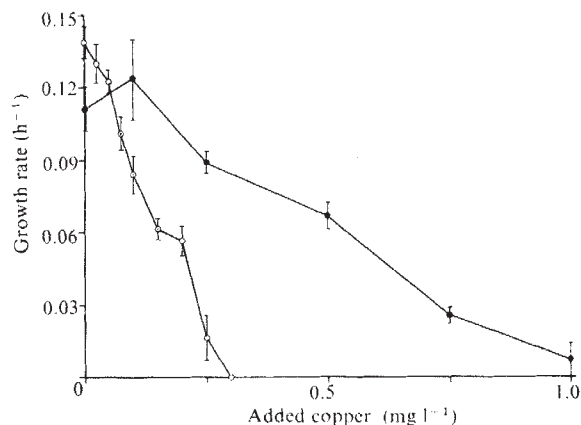


Fig. 1 The effect of copper added to the medium on the growth rate of tolerant (●) and non-tolerant (○) strains of *Chlorella vulgaris*. Cultures were grown axenically in defined medium with constant light (8,000 lx supplied by 10 40-W warm white fluorescent tubes) and temperature (24 ± 1 °C). The medium contained: 0.20 mM NaNO₃; 0.04 mM MgSO₄; 0.07 mM CaCl₂; 0.25 mM NaCl; 0.006 mM K₂HPO₄; 0.024 mM KH₂PO₄; 5.0 mM PIPES (piperazine-*N,N'*-bis [2-ethane sulfonic acid]) at pH 6.2; plus 0.01 ml l⁻¹ of each of the standard Bold's basal medium micronutrient solutions¹⁴ but without EDTA. The pH was 6.2 at the beginning and end of growth. Cultures were of 50-ml volumes in 250-ml conical flasks and were aerated by shaking at 60 oscillations min⁻¹ on a reciprocal shaker. Copper was added as CuSO₄·5H₂O. Growth was followed as turbidity using an Evans Electroselenium nephelometer with a green filter (OGR1). Growth rates were computed from a least squares fit to the linear phase of the growth curves and are expressed as the reciprocal of the doubling times in h. Each point is the mean of three cultures; bars represent the standard errors of the mean.

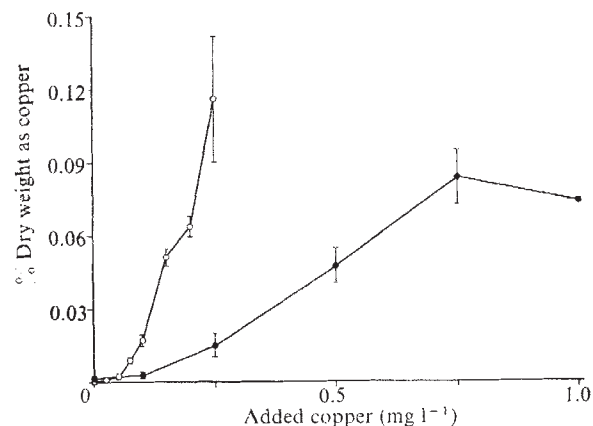


Fig. 2 The amount of copper, as percentage dry weight, accumulated by the tolerant (●) and non-tolerant (○) strains of *Chlorella vulgaris* against the amount of copper added to the medium. Stationary phase cells were centrifuged at 500g for 30 min, washed three times with 50-ml volumes of distilled water, and filtered on to washed and pre-weighed Gelman membrane filters (0.45 µm pore diameter). After drying at 70 °C for 24 h, filters were weighed to determine dry weight yield, boiled in 10 ml Aristar HNO₃ to near dryness, taken to 5 ml with distilled water, and analysed for copper with an Evans Electroselenium 240 atomic absorption spectrophotometer fitted with a Shandon Southern Instruments A3470 flameless attachment (carbon rod). Results are expressed as percentage dry weight as copper, computed as the amount of copper accumulated by the algae divided by the total dry weight yield of each culture, $\times 100$. At 0 mg l⁻¹ copper, the total yields of the two strains were equivalent. With increasing copper, the yields were reduced; for both strains, the minimum yields, at the highest copper concentrations, were 60% of those of the 0 mg l⁻¹ copper controls. Each point is the mean of three cultures, except those points at copper concentrations at which not all cultures grew, namely, the non-tolerant strain at 0.25 mg l⁻¹ copper (mean of two cultures) and the tolerant strain at 1 mg l⁻¹ copper (one culture). Bars represent standard errors of the mean.

reduced the growth rates of both strains, the tolerant strain was much less sensitive. A copper concentration of as little as 0.05 mg l^{-1} decreased the growth rate of the non-tolerant strain, and 0.30 mg l^{-1} was completely inhibitory. The tolerant strain, however, was not affected by 0.10 mg l^{-1} , and one of three cultures at 1.00 mg l^{-1} survived and grew.

Other reported effects of heavy metals on unicellular algae—increased lag period^{15–17}, increased cell size^{15,16,18} and decreased yield of cell number^{1,18} and dry weight¹⁹—were observed with both strains. The appearance of giant cells, up to 20 times the normal volume, reflects an uncoupling of growth from cell division; as pointed out previously^{15,18,20}, this phenomenon indicates that heavy metals may specifically inhibit division in algae. Also, early in the growth phase, dividing cells failed to separate, resulting in multicellular aggregates, which persisted until late log phase. Thus it seems that heavy metals also affect cell separation.

Figure 2 shows that the non-tolerant strain accumulated 5–10 times more metal at comparable external copper concentrations than did the tolerant strain. The growth rate of each culture as a function of the amount of copper accumulated by the algae is shown in Fig. 3. The lack of difference between the two strains implies that they respond identically to the same amount of cellular copper. Other growth parameters, for example, lag period and yield, are

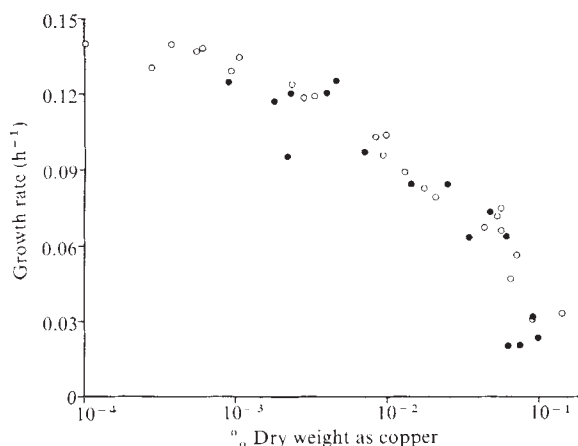


Fig. 3 The relationship between the growth rate of each culture and the amount of copper, as percentage dry weight, accumulated by the tolerant (●) and non-tolerant (○) strains of *Chlorella vulgaris* grown at various external copper concentrations. Growth rate is the reciprocal of the doubling time in h.

also identical in the two strains at equivalent cellular copper concentrations. Thus copper exclusion is likely to be the only mechanism of tolerance in this alga.

There have been a few reports of metal tolerant organisms that exclude metals. Micro-organisms commonly accelerate the vaporisation of mercury^{21,22}, although Davies¹⁸ reported that the mercury tolerance of the marine alga *Dunaliella tertiolecta* was primarily due to internal detoxification. Bryan and Hummerstone⁸ found a zinc-tolerant marine polychaete, *Nereis diversicolor*, to be impermeable to zinc, cadmium and manganese; copper tolerance in this animal may also result in part from decreased permeability²³. Other zinc-tolerant organisms, including the freshwater bryophyte *Scapania undulata*²⁴, and several species of freshwater filamentous algae (my unpublished results), also seem to exclude zinc.

Because algae form the base of many aquatic food chains, a mechanism of tolerance involving the exclusion of metal may be important to the ecology of polluted environments. A metal that is not accumulated by primary producers is less likely to be concentrated in higher trophic levels.

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- Bradshaw, A. D. *Nature* **169**, 1098 (1952).
- Antonovics, J., Bradshaw, A. D. & Turner, R. G. *Adv. ecol. Res.* **7**, 1–85 (1971).
- Russel, G. & Morris, O. P. *Nature* **228**, 288–289 (1970).
- Stokes, P. M., Hutchinson, T. C. & Krauter, K. *Can. J. Bot.* **51**, 2155–2168 (1973).
- Harding, J. P. C. & Whitton, B. A. *Br. phycol. J.* **11**, 417–426 (1976).
- Turner, R. G. in *Ecological Aspects of Mineral Nutrition of Plants* (ed. Rorison, I. H.) *Br. ecol. Soc. Symp.* **9**, 399–410 (Blackwell, Oxford, 1969).
- Ashida, J. A. *Rev. Phytopath.* **3**, 153–174 (1965).
- Bryan, G. W. & Hummerstone, L. G. *J. mar. biol. Ass. U.K.* **51**, 845–863 (1971); **53**, 839–857, 859–872 (1973).
- Hutchinson, T. C. & Stokes, P. M. in *Water Quality Parameters, ASTM STP* **573**, 320–343 (Am. Soc. for Testing and Materials, 1975).
- Brown, B. E. *Water Res.* **10**, 555–559 (1976).
- Turner, R. G. & Marshall, C. *New Phytol.* **70**, 539–545 (1971).
- Silverberg, B. A. *Phycologia* **14**, 265–274 (1975).
- Brown, B. E. *Freshwat. Biol.* **7**, 235–244 (1977).
- Nichols, H. W. & Boid, H. C. *J. Phycol.* **1**, 34–38 (1965).
- Stemann Nielsen, E. & Kamp-Nielsen, L. *Physiol. Pl.* **23**, 828–840 (1970).
- Nuzzi, R. *Nature* **237**, 38–40 (1972).
- Bartlett, L., Rabe, F. W. & Funk, W. H. *Water Res.* **8**, 179–185 (1974).
- Davies, A. G. *J. mar. biol. Ass. U.K.* **56**, 39–57 (1976).
- Greene, J. C., Miller, W. E., Shiroyama, T., Soltero, R. A. & Putnam, K. *Proc. Symp. on Terrestrial and Aquatic Ecological Studies of the Northwest, Eastern Washington State College, Cheney, Washington, March 26–27*, 327–336 (1976).
- Kanazawa, T. & Kanazawa, K. *Pl. cell. Physiol. Tokyo* **10**, 495–502 (1969).
- Tomomura, K., Maeda, K., Futai, F., Nakagami, T. & Yamada, M. *Nature* **217**, 644 (1968).
- Ben-Bassat, D., Shelef, G., Gruner, N. & Shuval, H. I. *Nature* **240**, 43–44 (1972).
- Bryan, G. W. in *Effects of Pollutants on Aquatic Organisms* (ed. Lockwood, A. P. M.) *Soc. exp. Biol., Sem. Ser.* **2**, 7–34 (1976).
- McLean, R. O. & Jones, A. *Freshwat. Biol.* **5**, 431–444 (1975).

Genetic variation in wild populations of rain-forest trees

IN the tropical rain forest, many tree species grow close together, and they are apparently equivalent in their edaphic requirements and spatially interchangeable. Most species occur at low density although they may be geographically widespread. No other plant community contains as many species of a single life form as do certain mixed dipterocarp forests of the aseasonal wet lowlands of Malesia^{1–3}. Such a population structure and level of diversity makes it hard to see how speciation has occurred. Spatial isolation of individuals and the abundant, normally hermaphrodite flowers simultaneously available for fertilisation in the canopy of the dipterocarp emergents suggest the possibility of self-fertilisation, but many of the understorey species are dioecious. As part of a comparative study of the reproductive biology of rain forest tree species, intra-specific genetic variation of isozymes has been demonstrated for the first time in an emergent and an understorey species of rain forest trees. Polymorphism is common in both species and there is evidence for short-range spatial heterogeneity in gene frequency and also in leaf morphology.

The species studied were *Shorea leprosula* Miq. an emergent species, and *Xerospermum intermedium* Radlk. an understorey species. *S. leprosula* is a dipterocarp which occurs widely in Thailand, Sumatra and Malaysia. *X. intermedium* is a member of the Sapindaceae which includes *Nephelium lappaceum*, the rambutan. Except in Malaysia and Borneo, where it is common on well-watered slopes, its distribution and abundance throughout South-East Asia is not yet known. Samples were collected from three sites, mostly from the Pasoh Forest Reserve which is in the southern part of Peninsular Malaysia.

Electrophoretic analysis of aqueous extracts of leaves was performed in starch or acrylamide slab gels and, after separation, gels were treated to identify the presence of esterases, leucine amino-peptidase, glutamic-oxalacetic transaminase, acid and alkaline phosphatase and also an

unidentified enzyme which proved useful in *S. leprosula*. Initial difficulties in resolving enzymes, especially in *X. intermedium*, were overcome by incorporating polyvinyl pyrrolidone, diethyldithiocarbamate and mercaptoethanol in the extraction buffer at pH 8.6; technical details will be reported elsewhere. Control extracts were incorporated in all runs and the R_F values were adjusted to the value in the reference sample to allow comparisons between gels. In most systems there was evidence of age-related changes in gene activity and so comparisons are confined within single age groups, for example, seedlings or mature trees, and refer to the presence or absence of a band at defined R_F positions.

In addition to the electrophoretic evidence the study has included for *X. intermedium* an analysis of variation in leaf morphology between trees in relation to their distance apart. Evidence of a somewhat different kind has been provided by comparisons of electrophoretic patterns in ten species of *Shorea* with morphological differences between them.

In *S. leprosula* in which 80–250 individuals were sampled per enzyme system, there is striking evidence of genetic polymorphism at alternative R_F positions. Table 1 shows that more than half the total number of positions, pooled over all systems, show variation while the frequency of the rarer state is 0.10 or above. This indicates high levels of polymorphism and hence of heterozygosity. We cannot yet determine the number of loci involved. Tests of independence of presence or absence of bands for all possible pairs generally indicate independence but not always so, probably due to determination of more than one band by a single locus. Column *B* of Table 1 allows for such correlations and the actual number of loci probably corresponds more closely to the bands referred to in column *B* than to those in *A*. Thus *S. leprosula* resembles many other outbreeding species in being highly heterozygous.

Table 1 Combined record of variation in *Shorea leprosula* shown by electrophoretic systems

	<i>A</i> Total no.	<i>B</i> Adjusted no.
R_F positions	40	36
% with variable band presence	55	50

In *X. intermedium* fewer systems were studied and the number of bands per system was less than in *S. leprosula*. In samples from 56 trees, six putatively distinct loci were identified and two of these, both determining esterases, were polymorphic. Comparisons from wild individuals as well as test crosses suggested the occurrence of a pair of alleles at each locus, one active and the other null, with a frequency of 0.97 and 0.49 for the active alleles in the 'fast' and 'slow' positions, respectively. Each locus conformed to the Hardy-Weinberg distribution and each apparently segregated independently of the other. Thus the genetic situation in this species seems to be essentially the same as in *S. leprosula*.

Spatial heterogeneity was evident in *S. leprosula*. Samples derived from groups of trees separated by approximately 700 m are likely to differ significantly at one or more R_F positions. There is also evidence that the degree of difference is correlated with distance apart and this may be related to limited dispersal of pollen and fruit. For *X. intermedium* there are similar indications; heterozygotes often occur between or close to alternative homozygotes. In this species, variation in leaf morphology has been studied in samples of generally ten leaflets from 68 trees growing in an area 600 m². Analysis of variance within

and between trees for leaf length, breadth, vein number, ratio of vein number to length and ratio of leaf length to breadth revealed highly significant heterogeneity between trees. The distance between any pair of trees can be computed from the coordinates of a rectangular grid applied to a map of the trees' location. The regressions of differences for all possible pairs for the leaf characters on distance apart proved highly significant ($P < 0.001$). How far genetic variation contributes to the variation in the leaf characters is not known but this evidence is not inconsistent with that from the electrophoretic study.

The electrophoretic patterns of 10 species of *Shorea* were compared for esterases, glutamic-oxalacetic transaminase and alkaline phosphates. An index of similarity (*I*) refers to the ratio of bands in common to total bands identified*. Combining the systems the total band number ranges from

Table 2 Values of *I* based on gel-band patterns in comparisons of species of the genus *Shorea* with *S. leprosula*

Species	Total no. of bands	<i>I</i>
<i>S. curtisii</i>	32	0.500
<i>S. parvifolia</i>	33	0.424
<i>S. acuminata</i>	29	0.413
<i>S. dasyphylla</i>	29	0.275
<i>S. macroptera</i>	31	0.258
<i>S. lepidota</i>	27	0.222
<i>S. macrantha</i>	29	0.137
<i>S. hemsleyana</i>	29	0.103
<i>S. ovalis</i>	35	0.000

27 to 35. Table 2 shows the paired comparisons between *S. leprosula* and nine other species. There is good agreement between *I* value and taxonomic affinity³. Thus *S. leprosula*, *S. parvifolia* Dyer and *S. curtisii* Dyer ex King are taxonomically close and agree in having high *I* values. Indeed for *S. leprosula* and *S. curtisii* we have the highest *I* value of 0.5 and for these two species hybrids have been identified in the field. Comparison with *S. ovalis* (Korth.)Bl. provides a zero value consistent with its taxonomic position in a separate section of the genus. It is likely that such electrophoretic evidence will make a valuable contribution to the taxonomy of dipterocarps and probably other groups where specific distinctions are often hard to define.

In *S. ovalis*, a tetraploid, 40 seedlings were collected from the base of four possible parent trees and examined by electrophoresis; altogether 36 R_F positions were available for comparison and of these only two, both esterases, were variable. The low level of variation in this species may be related to self-fertilisation (H. T. Chan, work in preparation).

We infer from the evidence reported here that both the emergent species *S. leprosula* and the understorey species *X. intermedium* have an outbreeding system, combined possibly with short-range heterogeneity in gene frequency which may be related to predominantly short-range pollen and fruit dispersal. The latter conditions contribute to physical isolation between populations and hence may promote speciation. Our findings are being compared with evidence relating to cytology, fertility, pollination mechanisms and breeding structure to set the evolutionary problems of rain forest trees in better perspective.

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¹ Ashton, P. S. *Oxford Forest Mem.* 25, (1964).

² Whitmore, T. C. *Tropical Rain Forests of the Far East* (Oxford University Press, Oxford 1975).

³ Symington, C. F. *Malay. Forest Rec.* 16, 2 (1943).

⁴ Sarich, V. M. *Nature* 265, 24 (1977).

Freezing avoidance by deep supercooling in hydrated lettuce seeds

DRY seeds of several species survive exposure to extremely low temperatures, including immersion in liquid air for up to 60 d (refs 1–3), but most young seedlings are very susceptible⁴. Fully hardened twigs of several northern tree species tolerate freezing in liquid nitrogen (-196°C) (ref. 5), and deep supercooling seems to be an important mechanism for the avoidance of freezing in both xylem ray parenchyma and flower buds of many other hardy but less cold resistant, deciduous, woody species^{6,7}. Resistance to freezing stress in seeds has been suggested to be related to water content^{1,4,8}, but little attention has been paid to either the relationship between stage of germination and degree of freezing resistance or the nature of the resistance. We report here that hydrated lettuce seeds (achenes) can avoid injury by deep supercooling and that the level of resistance can be determined precisely by differential thermal analysis (DTA). Furthermore, we show that the

deep supercooling is dependent on the intact structure of the endosperm.

Experiments were conducted with seed of Grand Rapids lettuce. Moisture content was controlled by leaving seeds to imbibe water for specific intervals; by leaving them to imbibe in different concentrations of polyethylene glycol (molecular weight 6,000, PEG 6000) for specific intervals, and by storing them at controlled relative humidities in closed vials on top of various concentrations of H_2SO_4 . Moist seeds were dried rapidly between filter papers and samples were prepared for freezing and for determination of water content. Seeds that had imbibed in polyethylene glycol were washed three times with distilled water before preparation. Dry weight was determined after drying at 105°C for 24 h. Moisture content was calculated as percentage water of fresh weight. Supercooling nucleation points were determined by DTA (ref. 7) with an automatically controlled, liquid- N_2 -cooled Cryoson freezing system, with a constant freezing rate of 20°C per h. Exotherms were recorded on potentiometric recorders, at 0.5 mV, connected to the samples with 0.5 mm copper-constant thermocouples. Between 1 and 10 seeds were used with each recording channel. Samples (10 to about 100 seeds) for survival tests were removed from the freezer at predetermined temperatures and kept at 2°C for 2–4 h before ability to germinate was tested in light at 24°C . Seeds were counted after 3 d and the percentage of germinated and growing seeds was used as a criterion for viability.

Lettuce seeds with 5–13% water content were not injured by freezing to -196°C , but with 13–16% moisture, the threshold of freezing tolerance declined to -40°C (Fig. 1a). No exotherms were detected by DTA in either case but two types of exotherms were found in intact seeds

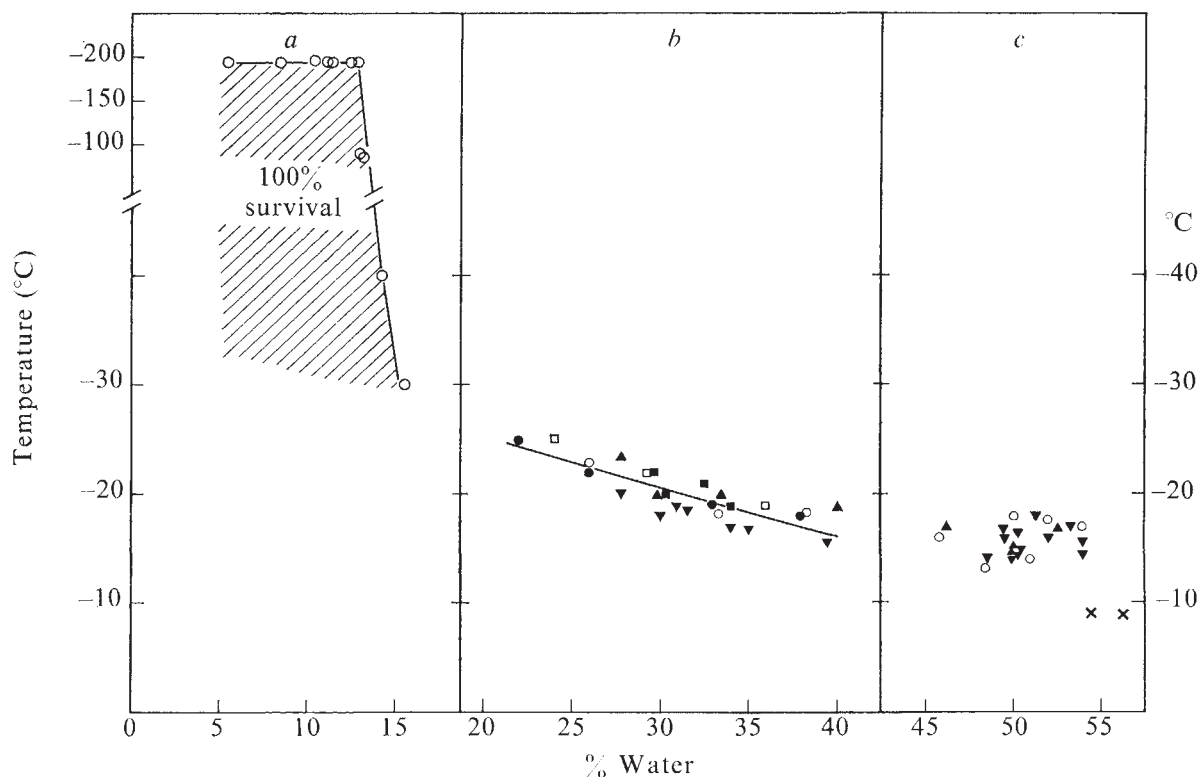


Fig. 1 Relationship between seed water content and hardiness in Grand Rapids lettuce seeds. *a*, Limited for 100% survival of seeds stored at 18°C or at 4°C in controlled humidities for 10–14 d. Hardiness at -196°C was tested by keeping the seeds in liquid nitrogen for 30 min. *b*, The mean temperature of the secondary exotherms in seeds imbibed at 30°C in various concentrations of PEG 6000 (0.06–0.12 molal). Open symbols: 5 min red (R) light, $4.2 \mu\text{W cm}^{-2}$ at 660 nm, given after 2 h imbibition in darkness; closed symbols: darkness. $\square$, $\blacksquare$, 12 h imbibition; $\circ$, $\bullet$, 24 h; ∇ , 72 h; $\blacktriangle$, 96 h. Regression equation: $y = -0.43x + 33.9$, $r = -0.84$, $P < 0.001$. *c*, Mean temperature of secondary exotherms in seeds imbibed at 30°C in water for 4–72 h. $\circ$, R; $\blacktriangle$, R plus 5 min far-red (FR), $24 \mu\text{W cm}^{-2}$ at 730 nm; $\blacktriangle$, darkness; $\times$, germinated seeds.

containing more than 20–25% water (Fig. 2). The first appeared as a single peak when the temperature of the sample was $-10 \pm 2^\circ\text{C}$ regardless of the number of seeds in the sample, indicating that the nucleation point for this peak is remarkably uniform. This exotherm is not related to the injury of intact seed and we suggest that it represents freezing of extracellular or bulk water in the outer layers, similar to extracellular freezing of other plant tissue⁷. Each seed frozen showed one deep supercooling exotherm. These secondary exotherms represent the killing point of individual seeds. In seeds imbibed in PEG 6000 the mean exotherm temperature was linearly correlated ($r = -0.84$, $P < 0.001$) with the moisture content within the limits of 20–40% (Fig. 1*b*). A plateau was reached at 40–50% moisture where the mean exotherm temperature of intact seeds remained at $-16 \pm 2^\circ\text{C}$ ($r = 0.004$), irrespective of imbibition time (4–72 h). There was no effect of red (5 min, $4.2 \mu\text{W}$

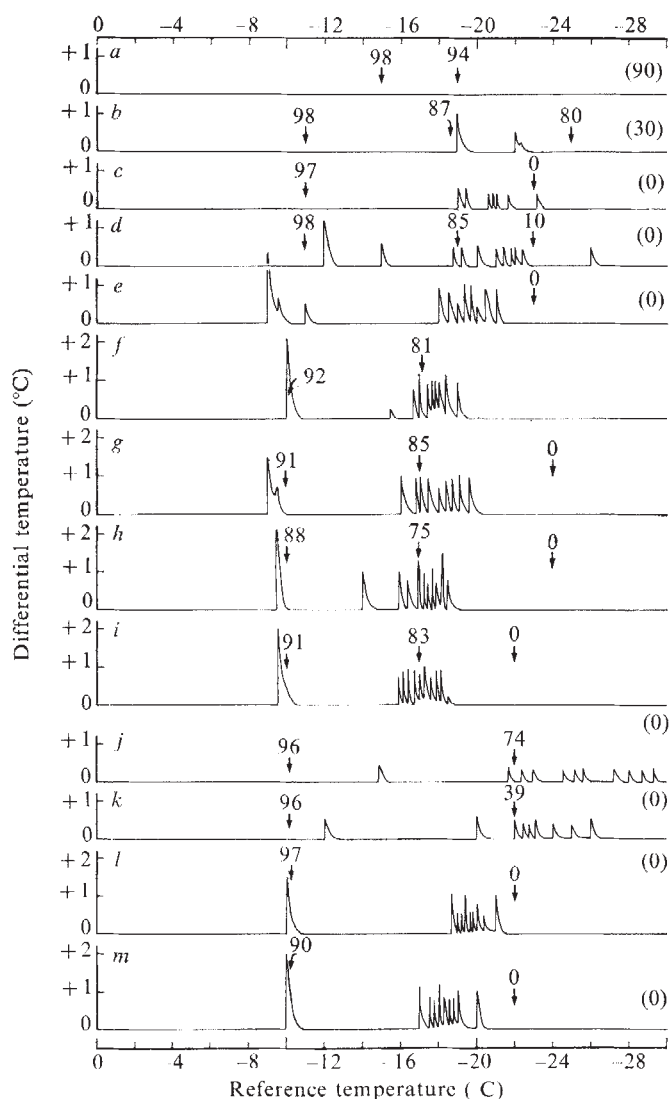


Fig. 2 Differential thermal analysis of lettuce seeds with moisture contents at various levels. Ten seeds in each sample. Figures and arrows show the percentage of survival (germination) in samples removed at temperatures indicated, those in parenthesis show survival of samples frozen down to -35°C . Freezing rate 20°C per h in all cases. To obtain the various moisture levels seeds were imbibed at 30°C in darkness either in water for various times (*a-i*) or in various molal concentrations of PEG 6000 (*j-m*) for 24 h. *a*, 0 or 5 min imbibition, 5.7–16% water content; *b*, 10 min, 20%; *c*, 20 min, 26%; *d*, 30 min, 32%; *e*, 60 min, 41%; *f*, 4 h, 49.7%; *g*, 8 h, 49.5%; *h*, 12 h, 48.8%; *i*, 24 h, 51.2%; *j*, 0.12 molal, 21.9% water; *k*, 0.10 molal, 26.0%; *l*, 0.08 molal, 33.3%; *m*, 0.06 molal, 38.2%.

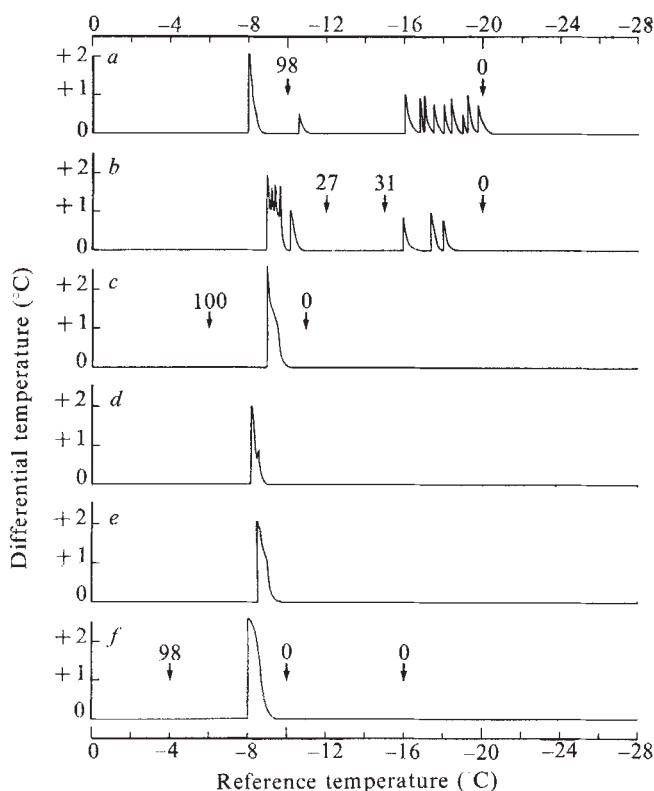


Fig. 3 Differential thermal analysis of lettuce seeds. *a*, Intact seeds; *b*, seed coats removed; *c*, excised embryos; *d*, endosperms; *e*, intact seeds stuck four times with a needle; *f*, germinated seeds. Figures and arrows are as in Fig. 2. Seeds coats and endosperm were removed with a scalpel under a stereoscope in light after an imbibition at 30°C for 16 h. Seeds stuck with a needle were imbibed in water for 4 h at 30°C . There were ten seeds or embryos in each sample.

$\text{cm}^{-2} \text{nm}^{-1}$ at 660 nm) or far red (5 min $24 \mu\text{W cm}^{-2} \text{nm}^{-1}$ at 730 nm) irradiation on the position of the secondary exotherms up to 8 h after exposure of seeds.

The freezing pattern changed considerably, however, after disruption of the endosperm–seed coat envelope. As soon as the radicle emerged the secondary exotherms disappeared and the first exotherm represented the killing point of the seed (Fig. 3). Similarly, embryos excised from the seeds after imbibition for 2–12 h produced only one exotherm and did not survive below this nucleation temperature (Fig. 3). The occurrence of secondary exotherms depends on the intact structure of the endosperm. If fruit coats were excised carefully without injury to the endosperm envelope, embryos did supercool as detected by the presence of secondary peaks at the nucleation points, but if the endosperm was damaged, for example with a needle, deep supercooling ceased and death was coincident with the first peak (Fig. 3).

Therefore the integrity of the endosperm envelope seems to facilitate deep supercooling which concurrently imparts freezing avoidance to -12° to -18°C in intact, imbibed lettuce seeds. Deep supercooling of lettuce seeds is not, however, an exclusive property of live seeds: it also occurred with intact, imbibed seeds which had been killed by low or high temperatures. Work with apple stems has shown that intact dead twigs yield exotherms if rehydrated to 20% water but not if ground to a fine powder⁹.

The mechanism by which the intact endosperm imparts freezing avoidance to the embryo is not clear. Perhaps disruption of the endosperm facilitates intimate contact of relatively pure water in the coats with the water in the embryo, thus providing ice crystal formation in all parts of the seed at the first exotherm. Germinated

seeds contain slightly more water than imbibed non-germinated seeds, and perhaps endosperm injury increases embryo water content. But even embryos excised from seeds imbibed in 0.10 molal PEG 6000 and kept in 0.12 molal PEG 6000 during freezing did not yield secondary exotherms.

The mechanism of freezing resistance in lettuce seeds seems to resemble those found in other tissues and species at various stages of cold hardiness. We suggest that lettuce seed offers a useful system for the study of mechanisms of freezing tolerance and avoidance.

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- ¹ Kieselbach, T. A. & Ratcliff, J. A. *Nebr. Agric. Exp. St Bull.* 163, 1-16 (1918).
- ² Lipman, C. B. & Lewis, G. N. *Plant Physiol.* 9, 392-394 (1934).
- ³ Lipman, C. B. & Lewis, G. N. *Plant Physiol.* 11, 201-205 (1936).
- ⁴ Levitt, J. *Responses of Plants to Environmental Stresses* (Academic, New York, 1972).
- ⁵ Sakai, A. *Nature* 185, 392-394 (1960).
- ⁶ George, M. F. & Burke, M. J. *Curr. Adv. Plant Sci.* 8, 349-360 (1976).
- ⁷ Burke, M. J., Gusta, L. V., Quamme, H. A., Weiser, C. J. W. & Li, P. H. *A. Rev. Plant Physiol.* 27, 507-528 (1976).
- ⁸ Lockett, M. C. & Luyet, B. J. *Biodynamica* 7, 67-76 (1951).
- ⁹ Quamme, H., Weiser, C. J. & Stushnoff, C. *Plant Physiol.* 51, 273-277 (1973).

Guard cell malic acid metabolism during stomatal movements

THE mechanism by which stomata open and close has long been a puzzle, but only now are we beginning to make major advances in our understanding of their metabolism. It has been concluded that guard cells can fix CO_2 and incorporate it into starch¹⁻³. It has been shown that the bulk of, if not all, the CO_2 fixed by guard cells is converted to oxaloacetic acid (OAA) by phosphoenolpyruvate carboxylase and the OAA, in turn, is reduced to malic acid^{3,4}. Some amination of OAA producing aspartate has also been detected⁴. Although there is indirect evidence suggesting that aspartate and/or malate can provide carbon for starch synthesis in the guard cells⁴ direct evidence for this is lacking. We have investigated some characteristics of guard cell CO_2 fixation which directly results in organic acid formation and we also have direct evidence that malic acid is a precursor of starch formation.

Microautoradiography showed that guard cells in epidermal strips of *Commelina communis* L. were able to fix CO_2 in the light or the dark irrespective of whether stomata were open or closed. Although some localisation of ^{14}C could be detected in the region of the epidermal cells the label was concentrated within the stomatal complexes. These observations were confirmed by CO_2 fixation kinetics experiments. Figure 1 shows similar rates of ^{14}C uptake by epidermal tissue of *C. communis* when floated on a $\text{NaH}^{14}\text{CO}_3$ solution in the light or the dark. Slightly greater uptake rates of ^{14}C were obtained in the light when epidermal tissue of *C. diffusa* was cut into small pieces and immersed in $\text{NaH}^{14}\text{CO}_3$ solution⁴.

Microautoradiography also showed that guard cells became more heavily labelled if the epidermis remained attached to the mesophyll. Whether this was due to some channelling of ^{14}C -labelled products from the mesophyll to the guard cells or whether the mesophyll in some way enhances guard cell CO_2 fixation is not known.

When L-(^{14}C)malic acid was fed to epidermal strips of *C. communis* while stomata closed from an aperture of $8\ \mu\text{m}$ in response to darkness, or while they opened from fully closed in response to CO_2 -free air and light, guard cell

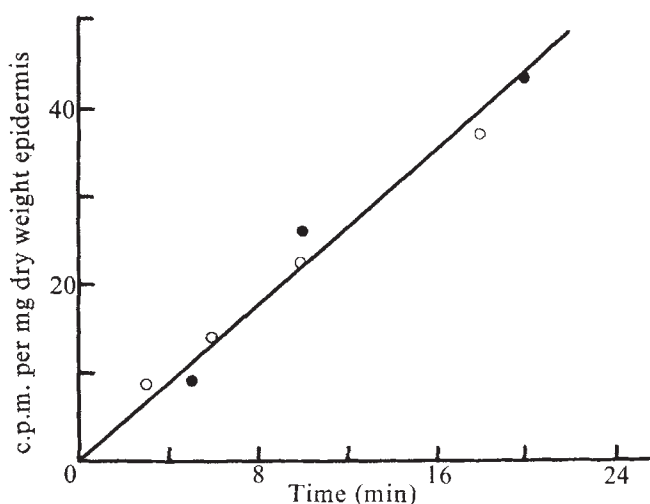


Fig. 1 CO_2 fixation kinetics of abaxial epidermal strips of *C. communis* in light (○) and darkness (●). Epidermal strips were floated, cuticle uppermost, on 3 ml 'suspension medium' containing 0.33 M sorbitol, 0.05 M tricine buffer (pH 8.0), 0.002 M sodium nitrate, 0.002 M EDTA, and 0.005 M potassium hydrogen phosphate. At zero time 0.2 ml of 0.05 M $\text{NaH}^{14}\text{CO}_3$ was injected into the suspension medium and the solution quickly swirled to mix, giving a CO_2 concentration of 4.5×10^{-5} M. At the indicated times tissue was immersed in 2.0 ml 20% trichloroacetic acid to stop the reaction and drive off excess $^{14}\text{CO}_2$. Radioactivity in the tissue was then determined by liquid scintillation counting.

starch became labelled in both cases (Fig. 2). Incorporation of label into starch, however, was greatest when the stomata were closing. Microautoradiographs also verified that label from malic acid was incorporated into starch, mainly located in areas corresponding to stomatal positions in the epidermis (blackened areas in Fig. 3). Thus, incorporation of ^{14}C from labelled malic acid into starch is not restricted to closing movements in epidermal strips. But, good correlations for guard cell malic acid accumulation⁵

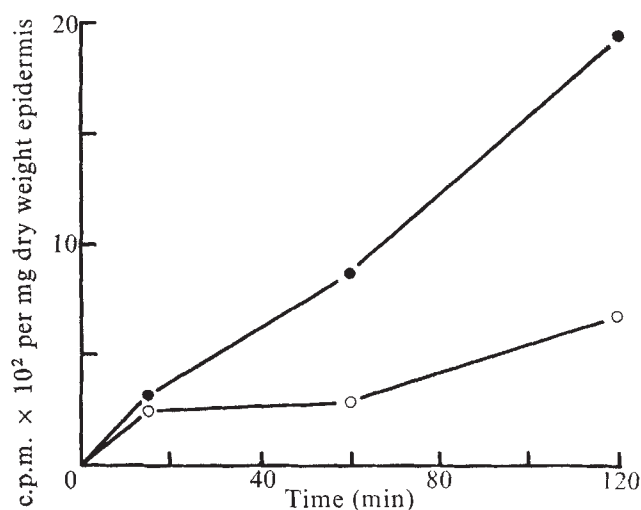


Fig. 2 Incorporation of ^{14}C from L-(^{14}C)malic acid into starch during 2-h exposure. The abaxial epidermis of *C. communis* was floated, cuticle uppermost, on 20 ml of 10 mM sodium citrate buffer containing 10 mM sodium chloride and 0.086 mg (25 μCi) $\text{U-}^{14}\text{C}$ -malic acid, pH 5.5. In one treatment the epidermal strips possessed closed stomata which were stimulated to open to $7\ \mu\text{m}$ after 2 h incubation in light and CO_2 -free air (○) and in the other, stomata were initially open to about $8\ \mu\text{m}$ and were made to close to about $4\ \mu\text{m}$ after 2 h incubation in darkness and normal air (●). At the times indicated, samples of tissue had their ethanol and water soluble products extracted leaving the insoluble starch and the radioactivity in the starch was determined by liquid scintillation counting.

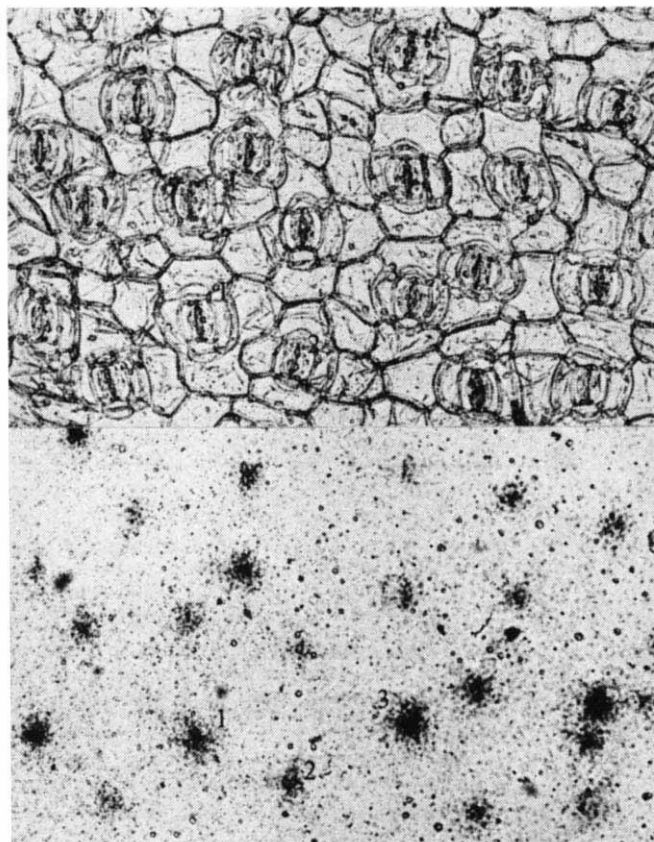


Fig. 3 Microautoradiograph of an epidermal strip of *C. communis* exposed to darkness and normal air for 2 h to close the stomata and after treatment as described in the legend to Fig. 2. Blackened areas located predominantly over the stomatal complexes indicate ^{14}C label in starch. (Stomata 1, 2 and 3 correspond to the numbered, blackened areas.) The dried epidermis was placed on a glass slide and a piece of Ilford Pan F film firmly taped on top with the emulsion in contact with the tissue. On top of the film was placed another slide and the whole was clamped together with screw-clips. After exposure for 29 d the piece of film was detached from the tape and tissue and developed in Kodak DX-80 developer.

and starch disappearance⁶ have been observed with stomatal opening. We have also found high levels of starch in guard cells of *C. communis* when stomata were closed; this level decreased as stomata opened although starch could still be detected at wide stomatal apertures. This suggests that there is a dynamic flux of carbon between malic acid and starch with the direction of starch production outpacing its breakdown when stomata are closing. Results of an investigation of pathways for malic acid-starch interconversions will be published elsewhere.

In conclusion we have established that malic acid is a precursor of starch synthesis in guard cells of *C. communis*. But, the mechanism of the control of guard cell CO_2 fixation and hence malic acid synthesis, and the control of the production of starch from malic acid in relation to stomatal movements, remain obscure.

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¹ Shaw, M. & MacLachlan, G. A. *Can. J. Bot.* 32, 784-794 (1954).

² Nishida, K. *Physiologia Pl.* 16, 281-298 (1963).

³ Willmer, C. M., Pallas, J. E., Jr & Black, C. C., Jr. *Pl. Physiol.* 52, 448-452 (1973).

⁴ Willmer, C. M. & Dittrich, P. *Planta* 117, 123-132 (1974).

⁵ Allaway, W. G. *Planta* 110, 63-70 (1973).

⁶ Meidner, H. & Mansfield, T. A. *Physiology of Stomata* 141-145 (McGraw-Hill, New York, 1968).

Orientation columns in macaque monkey visual cortex demonstrated by the 2-deoxyglucose autoradiographic technique

In the past fifteen years physiological studies of the primary visual cortex in higher mammals have provided evidence for two independent systems of functional subdivisions, ocular dominance columns and orientation columns¹. These two systems are closely related to two important functions of visual cortex: combining at a single-cell level the information that originates in the two eyes, and rearranging the spatial information from the lateral geniculate body so that cells after the initial stage of visual processing come to respond to specifically oriented lines in the visual field.

The ocular dominance columns have been demonstrated anatomically by three different staining techniques²⁻⁶. We describe here the use of a new method⁷ which makes possible the anatomical demonstration of the orientation columns.

The original evidence that cells are aggregated according to their response characteristics came from microelectrode recordings¹. In a penetration perpendicular to the cortical surface all cells are dominated by the same eye and all give optimal responses to the same stimulus orientation, whereas in an oblique or tangential penetration there is an alternation of the dominant eye, from left to right and back, and, at the same time, a series of regular changes in optimal stimulus orientation in steps of 10° or less, clockwise or counterclockwise. Reversals in direction of rotation occur sporadically, on the average every few mm, and the orderly sequences of small orientation shifts are occasionally broken by abrupt large shifts of up to 90° . A full cycle of either type, one eye and then the other or a rotation through 180° , generally requires a horizontal movement along the cortex of 1 mm or less⁴. The constancy of eye dominance and of optimal stimulus orientation during perpendicular penetrations indicates that the two sets of subdivisions are arranged perpendicular to the surface and the layers. Because of their cross-sectional shape in brain sections perpendicular to the surface, the subdivisions have been called 'columns', and a complete set of columns (left plus right eyes, or a full 180° rotation) is called a hypercolumn.

Inspection of cortical sections stained by conventional methods gives no hint of these vertical subdivisions. The only obvious segregation of cells is the horizontal system of layers, and this segregation has certain physiological correlates. Layer IVc, at about mid-cortical thickness, is the site of termination of the geniculate afferents and contains cells that differ in their physiological properties from cells in the other layers in two respects: like the geniculate afferents, they have no orientation preference; and they are almost all strictly monocular. In contrast, cells in the layers above and below IVc almost all show clear orientation specificity and about half are binocular, though any given cell is likely to respond best to one or the other eye.

In the last few years considerable progress has been made in working out the geometry of the columnar subdivisions. Three independent anatomical methods have made it possible to see the ocular dominance columns in layer IVc³⁻⁶ where they form a set of parallel bands which are for the most part straight, but in places form loops and whorls and occasionally show bifurcations and blind endings. The columns must therefore have the form of parallel sheets rather than being pillar-like. For the orientation columns,

microelectrode recordings, especially multiple parallel penetrations, likewise suggest an arrangement in parallel sheets, and the frequent reversals in direction of rotation would be compatible with a swirling pattern.

Until very recently no method has been available for demonstrating the orientation columns anatomically. Several years ago Sokoloff and his group⁷ developed a procedure for labelling active brain tissue. The method depends on the fact that brain cells, which use mainly glucose as a source of energy, take up 2-deoxyglucose as though it were glucose and metabolise it as far as 2-deoxyglucose-6-phosphate, but no further. This compound cannot easily pass out of the cell, and tends to accumulate. Physiologically active regions of brain may then be identified by the use of radioactively labelled deoxyglucose and autoradiography. This method was used by Sokoloff's group in monkeys in which one eye was stimulated during a 45-min period immediately following intravenous injection of deoxyglucose⁸. (The other eye was occluded in some monkeys and had been previously removed in others.) The result, a striking demonstration of the ocular dominance columns in the striate cortex, differed from the results obtained by the other anatomical techniques in demonstrating the columns through the full cortical thickness, rather than showing just the parts in layer IVc.

We have used the deoxyglucose method to reveal the orientation columns. Our procedure is based on that of Sokoloff and his group, to whom we are indebted for first-hand instruction in the method. We injected a lightly anaesthetised juvenile macaque monkey with ^{14}C -2-deoxyglucose, $150 \mu\text{Ci kg}^{-1}$ in $100 \mu\text{Ci ml}^{-1}$ saline, and then stimulated for 45 min by moving back and forth in front of the animal a black screen on which were pasted a set of irregularly spaced vertical white stripes. Stripe widths were $0.5-1^\circ$, and movement was $2-4^\circ \text{ s}^{-1}$. Both eyes were held open, protected by contact lenses, refracted at the screen distance (1 m) and aligned with a variable prism over one eye so that the foveas were superimposed. To be sure of the alignment and as a check on the state of the animal we also recorded from single binocular cells in striate cortex and superimposed the receptive fields.

At the end of the stimulus period the animal was given an additional dose of anaesthesia followed by a lethal dose of intravenous KCl. It was then decapitated, and the skull was cleaned of skin, gradually immersed over a 4-min period in liquid Freon-22 at -125°C , and stored at -80°C . Small blocks of brain were later sectioned at $20 \mu\text{m}$ in a cryostat at -26°C , and the sections were picked up on a cover slip and immediately dried at 98°C . These sections were then pressed against X-ray film for 2-3 weeks and the film was finally developed. Some of the sections used for autoradiography were also later stained for Nissl substance.

An autoradiograph made from a section perpendicular to the striate cortex is shown in Fig. 1. Vertical regions of dense label can be seen extending through the full thickness of cortex. About midway through the thickness is a dense uniformly labelled horizontal band. By comparing the autoradiographs with neighbouring sections stained for Nissl substance it can be shown that this band corresponds to layer IVc. This agrees with the observation from microelectrode recordings, that cells in layer IVc show no orientation specificity. No such uniform labelling was seen in IVc in the results of Sokoloff's group⁸ or in our experiments, when ocular dominance columns were labelled by this method.

The vertical labelled regions in Fig. 1 are on the average about 0.6 mm apart and occupy a considerable fraction of the repeat distance, as expected from the fact that each cell responds not just to a single orientation but to a range of orientations to either side of the optimum, with some cells highly selective and others less so. For example, in a sharply tuned cell the orientation that evokes a half-

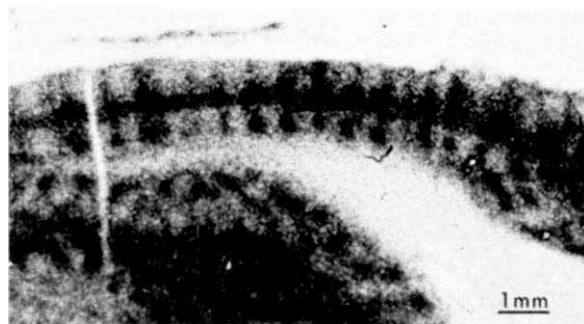


Fig. 1 Autoradiograph of a ^{14}C -2-deoxyglucose section through monkey striate cortex, perpendicular to the surface. The stimulus consisted of moving vertical, irregularly spaced white stripes presented to the entire visual field of both eyes for 45 min. Labelled regions are vertical in cross section, about 0.6 mm apart, and extend through the full cortical thickness. Layer IVc, situated at about mid-depth and identified from neighbouring Nissl-stained sections, is uniformly labelled, as expected from the absence of orientation specific cells in that layer.

maximal response may be $10-15^\circ$ from the optimal. The widths of the labelled regions must depend on many factors including the sharpness of tuning curves (response plotted against stimulus orientation), and the relationship between cell activity and deoxyglucose uptake, and between uptake and the grain density of the autoradiographs.

A tangential section through the dorsolateral surface of the occipital lobe is shown in Fig. 2. The white matter is grazed in two places which appear as pale ovals. These are surrounded by layers VI and V, cut almost tangentially, and here the labelled regions can be seen face-on, forming a complex pattern of rings, loops, and branching stripes. Their separation is strikingly constant, averaging roughly $570 \mu\text{m}$. Only in a few places is there a suggestion of parallel stripes. Surrounding this area is layer IVc, which is again uniformly labelled, and just outside IVc is IVb, where the aggregations of label are particularly dense. Layers II and III are more lightly labelled but also show distinct aggregations. (The dense bands at the extreme left, perpendicular to the surface, are microtome knife artefacts.)

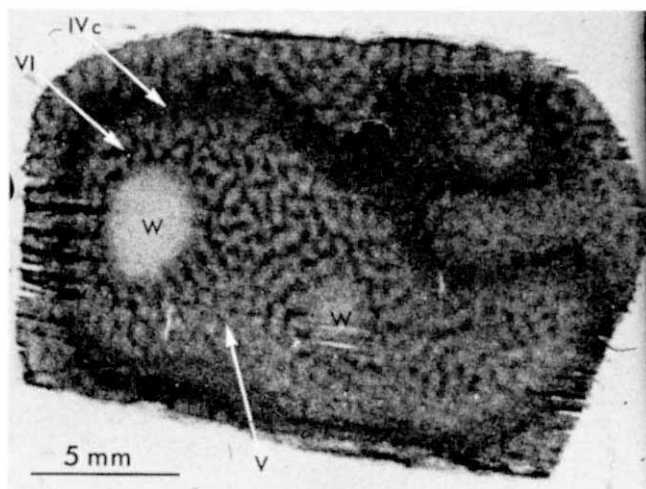


Fig. 2 Autoradiograph of a tangential section through the dorsolateral surface of the occipital lobe (striate cortex) of the same monkey. Dark areas are radioactively labelled. The section grazes white matter (W) in two places, which are seen as pale ovals. Surrounding these are densely labelled sets of orientation columns in layer VI. Outside this is layer V, lightly labelled, and then IVc, which is uniformly labelled with no hint of columnar subdivisions. The upper layers again show densities related to columns. (Dense bands perpendicular to the surface at the extreme left are artefacts produced by the microtome knife.)

More superficial sections from this block, tangent to the upper layers (II–III) rather than to white matter, show an almost identical pattern, as expected from the fact that the labelled regions are perpendicular to the surface.

So far we have examined too few brains to be sure that the pattern of the orientation columns is always as complex in form as that shown here. Ocular dominance columns were examined in the same region as that shown in Fig. 2, by transneuronal autoradiography following an injection of ^3H -proline into one eye two weeks before the deoxyglucose experiment. These showed a more regular pattern of stripes, with a spacing only slightly coarser than that of the orientation hypercolumns ($770\ \mu\text{m}$ compared with $570\ \mu\text{m}$). There was no obvious tendency for the two sets of columns to be related in any simple way: they were not consistently orthogonal, and were certainly not parallel. This result will be published separately⁹.

The anatomical demonstration of the orientation columns provides still another verification of the columnar organisation of the striate cortex. It is reassuring to find such agreement between morphology and physiology, and unusual to find the physiology actually leading the way to a structural description.

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- ¹ Hubel, D. H. & Wiesel, T. N. *J. Physiol. Lond.* **160**, 106–154 (1962); **165**, 559–568 (1963); *ibid.* **195**, 215–243 (1968); *J. comp. Neurol.* **158**, 267–294 (1974).
- ² Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **158**, 295–306 (1974).
- ³ Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **146**, 421–450 (1972).
- ⁴ Wiesel, T. N., Hubel, D. H. & Lam, D. M. K. *Brain Res.* **79**, 273–279 (1974).
- ⁵ Hubel, D. H., Wiesel, T. N. & LeVay, S. *Phil. Trans. R. Soc. B* **278**, 377–410 (1977).
- ⁶ LeVay, S., Hubel, D. H. & Wiesel, T. N. *J. comp. Neurol.* **159**, 559–576 (1975).
- ⁷ Sokoloff, L. in *Brain Work. The Coupling of Function, Metabolism and Blood Flow in the Brain* (eds Ingvar, D. H. & Lassen, N. A.) 385–388 (Academic, New York, 1975).
- ⁸ Kennedy, C. *et al. Proc. natn. Acad. Sci. U.S.A.* **73**, 4230–4234 (1976).
- ⁹ Hubel, D. H., Wiesel, T. N. & Stryker, M. P. *J. comp. Neurol.* (in the press).

Instability of the eye in the dark and proprioception

THE extraocular muscles of most mammals contain stretch receptors which are not necessarily in the form of muscle spindles. The cat, for example, has no muscle spindles, but the oculomotor muscles contain structures, with spiral endings, that are in parallel with the muscle fibres and respond to stretch¹. Most proprioceptive fibres run extraorbitally in the ophthalmic branch of the Vth nerve^{2,3}. The cell bodies of these fibres seem to be located in the mesencephalic root of the Vth nerve⁴. There is electrophysiological evidence of proprioceptive projections to the cerebellum^{5,6}, superior colliculus^{7,8} and visual cortex⁹. The function of ocular proprioception is uncertain, although there is evidence that in man it may be an indicator of eye position in the dark¹⁰. In the cat it seems to have a role in maintaining the binocularity of single neurones in the visual cortex^{11,12}. Because most proprioceptive fibres run into the ophthalmic branch of the Vth nerve, we have investigated eye movements in the alert animal before and after section of this branch. We report here that, in the absence of visual and vestibular stimuli, the eye ipsilateral to the section becomes unstable and performs slow pendular oscillations. Asymmetries are observed in the horizontal vestibular nystagmus recorded in total darkness from the two eyes after unilateral section.

Cats chosen for their docility were anaesthetised with pentobarbital. A plastic cylinder was fixed to the skull and Ag–AgCl electrodes were implanted for recording horizontal movements of both eyes. The cat was trained to stay

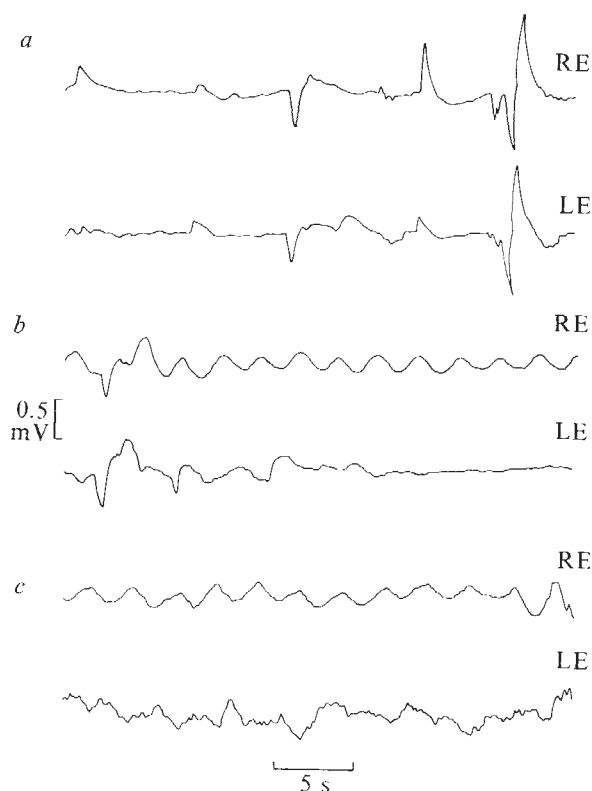


Fig. 1 *a*, Electro-oculographic a.c. recordings of horizontal movements of the right eye (RE) and left eye (LE) of a normal cat in the dark. *b*, The same, recorded 1 d after section of the ophthalmic branch of the right Vth nerve and *c*, 1 week after section of the ophthalmic branch of the left Vth nerve. Upward trace deflections indicate rotation of the eyes to the right, downward deflections, rotations to the left. The vertical bar corresponds to 5° horizontal eye rotation. Band pass: 0.1–20 Hz.

in a comfortable box with its head fixed by the plastic cylinder to a holder. The holder could either prevent any head motion or allow head rotations about a vertical axis. The cats were trained, over several days, to perform horizontal eye movements, tracking a visual target with the head either fixed or free to rotate horizontally. Eye movements were calibrated in each session by setting two pointers 30 cm from the eyes and displacing them 15° on either side of the cat's medial plane. The cat's attention was then called to either pointer while an experimenter checked the correctness of fixation, and the corresponding position of the recording trace was marked.

The cat box and the head holder were fixed to a table that could be oscillated sinusoidally about a vertical axis at various amplitudes and velocities.

When sufficient eye movements were recorded, the animal was again anaesthetised and the ophthalmic branch of the right Vth nerve was cut at the exit from the orbit or at the entrance into the semilunar ganglion. A small aperture was made into the parieto-temporal bone and the brain was lifted gently to reveal the three branches of the Vth nerve. Care was taken not to touch the oculomotor nerves. The operation was repeated on the left side after 2 or 3 weeks. After the experiments the animal was killed and the completeness of the section was checked.

We present here only results concerning eye movements in the dark, either spontaneous or elicited by head or body oscillations. The records of horizontal eye movements of normal cats that are awake in darkness contain periods with frequent spontaneous saccades, and periods with very few saccades, but with slow drifts mainly conjugate in the two eyes.

After section of the ophthalmic branch of the Vth nerve

no gross changes in the patterns of visually triggered eye movements were observed.

Seven out of nine cats displayed pendular movements of the eye ipsilateral to the section of the ophthalmic branch of the Vth nerve in the absence of visual stimuli (Fig. 1). The upper traces were recorded before the operation, the middle traces after section of the ophthalmic branch of the right Vth nerve and the bottom traces after further section of the ophthalmic branch of the left Vth nerve. The oscillations may have different amplitudes and frequencies in different cats, as comparison of Figs 1 and 2 (from two different cats) suggests. The frequency of the oscillations, however, was stable for each cat. The highest and lowest frequencies observed were 0.6 and 0.08 oscillations per s, the latter having a larger amplitude. There was no correlation between the occurrence of the oscillations and electroencephalogram activity, but the oscillations were more frequent when the cat was not performing frequent voluntary saccades. The two cats in which regular pendular movements were not observed were particularly restless even when deprived of visual and acoustic stimuli, and the rate of saccades was especially high in their eye movement recordings in the dark.

There were eye oscillations in most cats for at least 1 or 2 weeks after the operation. In three cats eye oscillations were observed a month after the second operation.

The patterns of eye movements induced by sinusoidal oscillations of the head or of the entire body became asymmetric in the two eyes after unilateral section of the ophthalmic branch of the Vth nerve. Alterations were induced in the eye ipsilateral to the section and consisted mainly of a decreased amplitude of the fast components of the vestibular nystagmus recorded in the dark. The patterns of vestibular nystagmus after unilateral section were equal in the two eyes when records were made in the light and were similar to those recorded before section. Details of these results will be reported elsewhere.

As controls, two cats underwent a complete unilateral operation except for the section of the nerves. Neither pendular motions in the dark nor asymmetries in the vestibular nystagmus of the two eyes were observed in these cats.

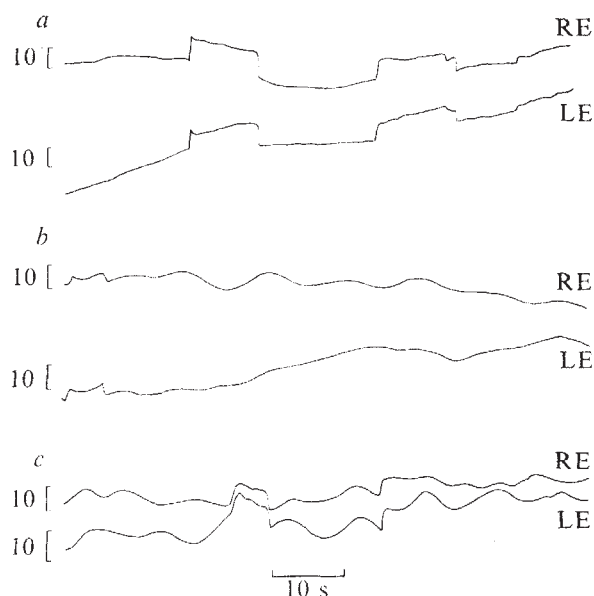


Fig. 2 *a*, Electro-oculographic d.c. recordings of horizontal movements of the right eye (RE) and left eye (LE) of a normal cat in the dark. *b*, The same, recorded 2 d after section of the ophthalmic branch of the right Vth nerve and *c*, 4 d after section of the ophthalmic branch of the left Vth nerve. Upward trace deflections indicate rotations of the eyes to the right.

The most obvious interpretation of the eye oscillations seen after section of the ophthalmic branch of the Vth nerve is that they result from the opening of the loop of the servo-system device responsible for the maintenance of eye position. Position errors can be signalled by two mechanisms: vision, indicating retinal error, and proprioception indicating relative stretch of the oculomotor muscles. It is not surprising that the interruption of the second source of information brings about oscillations of the eye in the dark.

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¹ Bach-y-Rita, P. & Ito, F. *J. Physiol., Lond.* **186**, 663 (1966).

² Batini, C. & Buisseret, P. *Arch. ital. Biol.* **112**, 18 (1974).

³ Batini, C., Buisseret, P. & Buisseret-Dalmas, C. *Brain Res.* **85**, 74 (1975).

⁴ Buisseret, P., Guérinand, J. P., Horcholle-Bossavit, G. & Tyc-Dumont, S. *J. Physiol., Paris* **65**, 369 A (1972).

⁵ Fuchs, A. & Kornhuber, H. H. *J. Physiol., Lond.* **200**, 713 (1969).

⁶ Baker, R., Precht, W. & Llinás, R. *Brain Res.* **38**, 440 (1972).

⁷ Abrahams, V. C. & Rose, P. K. *J. Neurophysiol.* **38**, 10 (1975).

⁸ Rose, P. K. & Abrahams, V. C. *Brain Res.* **97**, 95 (1975).

⁹ Skavenski, P. & Maffei, L. *Exp. Brain Res.* **28**, 421 (1977).

¹⁰ Skavenski, A. A. *Vision Res.* **12**, 221 (1972).

¹¹ Maffei, L. & Bisti, S. *Science* **191**, 579 (1976).

¹² Maffei, L. & Fiorentini, A. *Brain Res.* **105**, 73 (1976).

Leukocytes are consistently associated with degenerating embryos in IUD-bearing rhesus monkeys

IN spite of intensive research during the past 15 yr the precise mechanism whereby an intrauterine device (IUD) exerts its contraceptive action is unclear. There is wide agreement that the effect is intra- rather than extrauterine, and is probably elicited after arrival of the conceptus in the uterine cavity and before its attachment to the endometrium¹. The most plausible suggestion seems to be that the device acts as a foreign body and provides a sterile inflammatory reaction in the endometrium which in some unknown way renders the uterine cavity hostile to pre-implantation embryos^{1,2}. The presence of large numbers of inflammatory cells, consisting mainly of neutrophils and, to a lesser extent, macrophages, is a constant feature of the endometrial reaction in IUD-bearing women and other mammals. In some species there is a high positive correlation, in the presence of an IUD, between the concentration of leukocytes in the uterine fluid and the suppression of implantation (for example, in the rabbit³). So far, however, there has been no direct proof of a consistent association between leukocytes and unimplanted embryos in IUD-fitted primates. This paper provides evidence for such an association in the rhesus monkey.

Our study stems from the successful development of a method for the recovery of uterine embryos from normal monkeys⁴. It consists of flushing fluid through a large-bore, fenestrated needle inserted into the uterine lumen above the cervix and aspirating it through a second, fundal needle into a receiving syringe; it has a yield rate of about 50% of preimplantation embryos in animals examined between days 15 and 20 of the menstrual cycle.

Normal females without IUDs were first subjected to the flushing procedure for the recovery of uterine embryos and then fitted with a shortened tailless Margulies coil inserted surgically through the fundus. After two successive menstrual periods the animals were mated and flushed once, as before, between days 15 and 20 of the cycle. The contents of the flush was deposited immediately into a siliconised watch glass and searched under a stereomicroscope. Embryos were transferred to cold 2.5% glutaraldehyde in 0.1 M cacodylate buffer, photographed and processed for

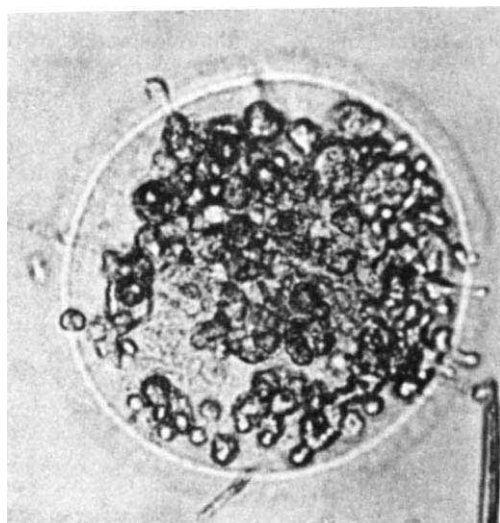


Fig. 1 Blastocyst from IUD-monkey (no. 476), showing leukocytes on the surface and apparently penetrating the zona pellucida ($\times 256$).

electron microscopical examination⁵. The remainder of the flush was centrifuged and the cellular composition of the pellet was determined by means of a haemocytometer and cytocentrifuge. During initial recovery attempts in IUD-animals, the flush was found to contain far more cells and debris than observed in non-IUD flushes (Table 1) and tended to clot, making the search for embryos difficult; heparin added to the receiving syringe reduced this tendency.

Sixteen females, from all of which embryos had been recovered in the non-IUD condition, have been flushed after being fitted with a device yielding six embryos in a total of 46 attempts (Table 1). Five of these embryos, the sixth being lost in processing, have been prepared and representative sections were examined by light microscopy and with an electron microscope. All five were considered to be abnormal or degenerating and each showed polymorphonuclear leukocytes both outside and within the embryonic tissue (Figs 1-3). In two of them macrophages were also present. In another, judged to be an azonal morula, all blastomeres were fragmenting, with leukocytes infiltrated between them. Two azonal embryos were blastocysts with

leukocytes present in the blastocoel cavities (Fig. 2). Another blastocyst possessed the zona pellucida and, again, leukocytes were found within the blastocoel and interspersed among cells of the inner cell mass. In two cases leukocytes were seen apparently passing through the zona pellucida (Fig. 3). The trophoblast layer showed irregular cells with disorganised cell junctions and an array of cytoplasmic membranous material not observed in normal monkey blastocysts⁵. The fifth embryo comprised only five or six shrunken cells within an intact zona. Electron microscopy revealed several polymorphs and macrophages in direct contact with embryonic cells. The polymorphs appeared to be typical, mature neutrophils and the macrophages had extensive plasma membrane invaginations. Occasional eosinophils were also present.

By contrast, of a total of three unfertilised ova and 30 embryos so far recovered from non-IUD monkeys, none had any polymorphs or other types of leukocyte attached to the surface, and only eight embryos were considered to be degenerating.

Cytological analysis of all IUD-flushes (irrespective of embryo recovery) showed that the number of cells varied from 4.37×10^5 to 2.39×10^6 , most of them being neutrophils (Table 1). Flushes from non-IUD fitted uteri gave

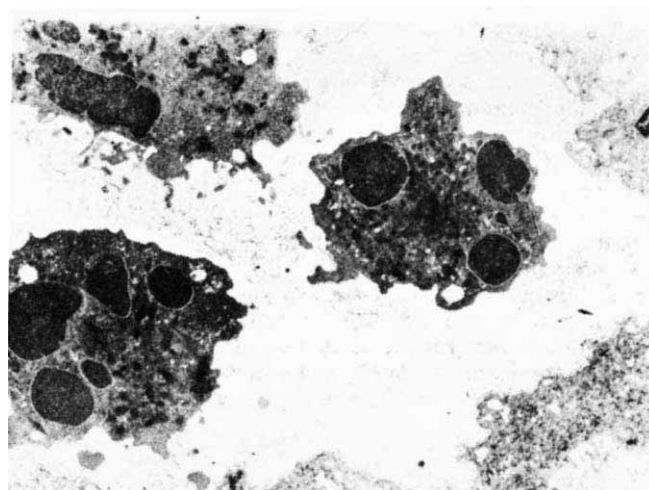


Fig. 2 Electron micrograph of a portion of an azonal blastocyst from an IUD-monkey (no. 616). Neutrophils are present in the blastocoel. T, Trophoblast layer ($\times 4,750$).

Table 1 Details of embryos and cells in uterine flushes from IUD- and non IUD-bearing rhesus monkeys

No. of female	Day of recovery*	Embryo stage	Total cell counts and differential distributions (%)						
			Totals	Neutrophils	Eosinophils	Monocytes	Lymphocytes	Macrophages	Endometrial
IUD flushes with embryo recovery (46 attempts in 16 females)									
476	16	5-6 cell, with zona	—	—	—	—	—	—	—
616	16	Blastocyst, azonal	5.08×10^5	65.7	6.7	8.4	0	7.2	12.0
617	19	Blastocyst, azonal	1.02×10^6	51.2	4.7	20.8	0.4	12.9	10.0
618	19	Morula, azonal	7.58×10^5	58.1	5.9	8.8	0.2	11.6	15.4
476	20	Blastocyst, with zona	9.39×10^5	33.2	11.2	10.7	1.9	27.6	15.4
613	15 (?)	Morula, azonal†	5.89×10^5	42.7	8.0	8.8	0	31.4	9.1
IUD flushes without embryo recovery (means $\pm$ s.e.; $n = 17$)									
—	—	—	$1.18 \pm 0.13 \times 10^6$	48.6 ± 3.6	5.0 ± 0.8	9.6 ± 1.1	1.4 ± 0.5	20.3 ± 2.5	15.3 ± 3.0
Non-IUD flushes without embryo recovery (means $\pm$ s.e.; $n = 7$)									
—	—	—	$2.32 \pm 0.62 \times 10^5$	11.3 ± 4.6	2.2 ± 1.4	6.0 ± 2.5	5.4 ± 2.0	0.8 ± 0.5	74.3 ± 9.1

*Day 1 counted as the first day of menstrual bleeding.

†Embryo lost in processing—no definitive identification made.

values between 1.44×10^4 and 3.92×10^5 (mean: $2.32 \pm 0.62 \times 10^3$), most being epithelial cells. By contrast, Joshi *et al.*⁶ reported no significant increase in polymorphs in the uterine flushings of IUD-fitted baboons. Their method of collecting and evaluating the flushes differed, however, substantially from our own. In particular, their use of centrifuge speeds of $2,500g$ is likely to cause leukocyte destruction, which can be avoided with speeds of less than $750g$ as employed by ourselves.



Fig. 3 Electron micrograph of the zona pellucida (Z) of an embryo from an IUD-monkey (no. 476). A leukocyte is seen passing through the zona into the blastocoel (B) ($\times 5,550$).

Our findings show that, in IUD-bearing rhesus monkeys, embryos can enter the uterine cavity at about the expected time after conception. They are, however, invariably abnormal or degenerating as well as invested with luminal leukocytes induced by the device. Also, compared with the yield in normal females (about 50%), far fewer embryos can be recovered at corresponding cycle days from IUD-fitted animals (about 13%).

It is feasible that the greatly increased cell content of the flush in IUD-monkeys may have made the detection of a recovered embryo impossible or that the embryo, when flushed out, was already too degenerated to be recognisable. It is also possible that in spite of normal fertilisation and early cleavage, embryos of IUD-fitted monkeys remain 'blocked' at or near the tubouterine junction and are therefore not recoverable by uterine flushing. The conclusion, implicit in our findings, that in monkeys with IUDs the blastocyst stage may be reached is indirectly supported by the finding of increased serum levels of human chorionic gonadotrophin in a proportion of IUD-bearing women examined during the second part of the menstrual cycle^{7,8}. Unfortunately no comparable data are available for rhesus monkeys.

Whether the embryos observed in our study were entirely normal before their investment and penetration by leukocytes is not known. It is possible that the highly cellular milieu of the uterine environment, perhaps derived from dead or dying leukocytes, is embryotoxic and that the main function of recently infiltrating live leukocytes is in the final destruction and removal of dying or damaged embryos. Nonetheless, the demonstrated association between degenerating uterine embryos and their investment with leukocytes in IUD-fitted monkeys is sufficiently constant and striking to suggest that it is more than coincidental.

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- Duncan, G. W. & Wheeler, R. G. *Biol. Reprod.* **12**, 143-175 (1975).
- Parr, E. L. & Shirley, R. L. *Fert. Steril.* **27**, 1067-1077 (1976).
- El-Sahwi, S. & Moyer, D. L. *Fert. Steril.* **22**, 398-408 (1971).
- Hurst, P. R., Jefferies, K., Eckstein, P. & Wheeler, A. G. *Biol. Reprod.* **15**, 429-434 (1976).
- Hurst, P. R., Jefferies, K., Eckstein, P. & Wheeler, A. G. *J. Anat.* (in the press).
- Joshi, S. G., Kraemer, D. C. & Chenault, C. B. *Contraception* **2**, 339-351 (1970).
- Landesman, R., Coutinho, E. M. & Saxena, B. B. *Fert. Steril.* **27**, 1062-1066 (1976).
- Belting, C. G., Cederqvist, L. L. & Fuchs, F. *Am. J. Obstet. Gynec.* **125**, 855-858 (1976).

Active thymus derived suppressor lymphocytes in human cord blood

A FOETUS carries antigens that are foreign to its mother, but the mother does not routinely reject it as an allograft. The fate of the foetus may thus depend on preventing or blocking the ability of the immune system of the mother to respond to alien foetal antigens. Clearly, this is a complex process which may involve several mechanisms¹⁻⁶. One mechanism implicates the active blocking of the immunological capacity of the mother to respond to her foetus^{5,6}. We reported previously that T lymphocytes taken from cord blood of human newborns inhibits their own mothers' lymphocytes from entering mitosis^{6,7}. We have studied the subpopulations of T lymphocytes in human cord blood and ascertained their ability to inhibit both the division of lymphocytes and the production of immunoglobulin by lymphocytes obtained from the mother. We report here that active T suppressor cells occur in cord blood, that these T suppressor cells bear IgG receptors; and that the T cells bearing IgG receptors inhibit both mitosis and immunoglobulin production by lymphocytes from the mother.

Blood (10-30 ml) was collected in heparin from the umbilical cords of healthy, singleton human males immediately after delivery but before expulsion of the placenta. Venous blood (25 ml) was collected in heparin from their mothers' arm within 1 h after delivery and from non-pregnant females and males (ages 21-43 yr, all healthy). These blood samples were the source of lymphocytes prepared by using Ficoll-Hypaque gradients. Division of the lymphocytes into T-cell enriched and B-cell enriched fractions was as described before⁷. The T cell population was over 97% pure, and the B cell population over 94% pure as judged by uptake of neutral red dye and expression of surface immunoglobulins⁷. T lymphocytes were separated into subpopulations bearing Fc IgG receptors (T_G) or IgM Fc bearing receptor cells (T_M) using the method of Moretta *et al.*^{8,9}. The purified T_G and the T_M subpopulations each contained less than 1% contaminating cells.

To study the effects of lymphocytes from each newborn on the growth of lymphocytes from his mother, equal numbers of cells from each mother and her baby ($1-2 \times 10^6$ from each) were combined and suspended in RPMI 1640 medium containing 20% foetal calf serum, antibiotics and glutamine (RPMI growth media) with phytohaemagglutinin P (PHA-P) (50 μ g per ml of medium). This concentration of PHA-P had been found to be optimal for stimulating lymphocytes from both the mother and baby¹⁰. When kept separate, both mothers' and newborns' cells divided equally well with an average viability of 60%-80% as determined by Trypan blue dye exclusion. After incubation for 3 d the cultures were treated with colchicine and the chromosomes prepared and stained as reported before^{7,11}. Cells from a male baby (positive for

Y chromosome) were always mixed with his mother's cells (lacking a Y chromosome).

Table 1 shows that when unseparated lymphocytes or enriched subpopulation of T_G cells from a newborn baby were cultured with equal numbers of lymphocytes from his mother, the vast majority of cells undergoing mitosis were of baby origin. In all six pairs studied in which the newborn's T_G cells were mixed with his mother's lymphocytes, a mean $\pm$ s.e.m. of $91.5 \pm 2.2\%$ of the total lymphocytes in mitosis were from newborns as determined by the presence of the Y chromosome (unfractionated T lymphocytes from cord blood gave a value of $90.7 \pm 1.4\%$), whereas 9% or less were from the mother ($P < 0.01$). In contrast, when T_G depleted fractions of cells were taken from a newborn and mixed with his mother's lymphocytes, only about half of the cells undergoing mitosis were of baby origin (Table 1). Thus in all six pairs involving T_G depleted cell populations, a mean of $49.3 \pm 2.3\%$ of the total lymphocytes in mitosis were from newborns as determined by the presence of the Y chromosome. When the T_M subpopulation of a newborn's cord blood lymphocytes were mixed with lymphocytes from his mother, roughly half of the cells undergoing mitosis were

of baby origin ($53.2 \pm 2\%$). Clearly, the subpopulation of T cells that suppressed mitosis of mothers' lymphocytes is that bearing Fc receptors for IgG. Further, these studies showed that lymphocytes collected from an infant's cord blood are primed to inhibit division of the mother's lymphocytes and do not need to be activated.

We then studied the effects of subpopulations of T lymphocytes obtained from each newborn on the ability of lymphocytes from his mother to make immunoglobulin. Experiments with polyclonal B cell differentiation induced by PWM had indicated both the need of T cell help and the optimal synthesis of immunoglobulin, which usually occurred by the seventh day^{9,12}. After culturing an enriched T cell population from a newborn baby with B lymphocytes from his mother, we found that significantly less immunoglobulin was synthesised than when the enriched T lymphocytes came from the mother. Hence, in all three pairs tested (Table 2), when T cells from the newborn were mixed with B cells from the mother, a 46% or greater reduction in immunoglobulin production (average $55.3 \pm 5.8\%$) occurred. These results could be influenced, in part, by the inability of 'immature' newborn T cells to provide sufficient help for adult B cells¹³. This is not the likely explanation for our results, however, as T cells from newborns or from adults provide equivalent amounts of help for synthesis of immunoglobulin by B cells of the newborn (Table 2). That T cells from a newborn apparently could not inhibit immunoglobulin production by its own B cells is consistent with our observation that lymphocytes from a newborn baby cannot abrogate the proliferation of lymphocytes from other newborns⁷. This result suggests that the newborn's lymphocytes, themselves, resist the inhibitory action for which they are responsible. Other studies showed that the suppression of immunoglobulin production by B cells from the mother resided in the T_G subpopulation of cells obtained from cord blood (Table 3). Hence, when equivalent numbers of a newborn's T_G lymphocytes were mixed with B cells from his own mother or from an alien mother, immunoglobulin synthesis was inhibited 63% or 65%, respectively.

Table 1 T_G subpopulation of T lymphocytes from human newborns suppresses mitosis of lymphocytes from their mothers

Case	Newborn lymphocyte subpopulation mixed with total maternal lymphocytes	Metaphase cells with Y chromosome (%)
P.A.	Total	94
P.A.	T_G	91
P.A.	T_G depleted	57
P.A.	T_M	59
C.L.	Total	88
C.L.	T_G	84
C.L.	T_G depleted	55
C.L.	T_M	52
L.O.	Total	89
L.O.	T_G	100
L.O.	T_G depleted	46
L.O.	T_M	49
R.O.	Total	89
R.O.	T_G	90
R.O.	T_G depleted	49
R.O.	T_M	53
W.H.	Total	96
W.H.	T_G	89
W.H.	T_G depleted	47
Mc.	Total	88
Mc.	T_G	95
Mc.	T_G depleted	42
	Expected result from 1:1 mixture	50

Lymphocytes from newborns and their mothers were obtained after Ficoll-Hypaque gradient centrifugation. The T cells were separated from these lymphocytes by E rosetting, and then distinct subpopulations of T_G or T_M cells were isolated on the basis of binding and rosetting with ox red blood cells (RBC) bearing the Fc portion of human IgG or IgM, respectively. Briefly, purified T cells were mixed with ox erythrocytes (ORBC) coated with rabbit IgG antibodies, pelleted and incubated on ice for a minimum of 10 min. Rosetting cells (T_G) were separated from non-rosetting cells (T_G depleted fraction) and purified by pelleting through two density gradients. The collected cell suspension contained less than 1% non-rosetting cells. RBC were removed by treatment with ammonium chloride buffer and the residual lymphocytes were suspended in 199 medium supplemented with 20% foetal calf serum, antibiotics and glutamine (199 growth medium), and pelleted. T_G enriched and T_G depleted cells were then resuspended in 199 growth medium and incubated overnight. T_G depleted cells were mixed with ORBC coated with rabbit IgM antibodies, pelleted and placed on ice for 1 h. The resulting rosettes purified on a Ficoll-Hypaque gradient were dissociated in a vortex mixer and purified human IgM at a concentration of $125 \mu\text{g ml}^{-1}$ added to block free IgM receptors. T_M cells were then separated from ORBC by density gradient centrifugation. Equal numbers of cells ($1-2 \times 10^6$) from male newborns and their mothers were mixed and cultured with PHA-P, $50 \mu\text{g ml}^{-1}$. After incubation for 3 d the cells were treated with colchicine, collected and stained with quinacrine hydrochloride (to mark the Y chromosome). Usually 50-100 cells in metaphase were examined from each culture for the presence or absence of the Y chromosome. In the culture conditions used, lymphocytes from mothers survived as well as lymphocytes from newborns and mean mitotic indexes of both pairs of cells were equivalent. Mixing adult (instead of newborn) male lymphocytes with lymphocytes from mothers (or other adult females) resulted in % of metaphase cells having a Y chromosome $\pm$ s.e.m. of $52 \pm 8\%$.

Table 2 T lymphocytes from human newborns suppress immunoglobulin production by lymphocytes from their mothers

Case	Source of lymphocytes	IgG made (ng per 1×10^6 B cells)	% Reduction
Y.O.	Mother Mother	116	
Y.O.	Mother Newborn	66	46
Y.O.	Newborn Newborn	62	
Y.O.	Newborn Mother	69	
L.O.	Mother Mother	498	
L.O.	Mother Newborn	232	54
L.O.	Newborn Newborn	58	
L.O.	Newborn Mother	47	
T.R.	Mother Mother	82	
T.R.	Mother Newborn	28	66
T.R.	Newborn Newborn	ND	
T.R.	Newborn Mother	ND	

Cells from newborns and their mothers were placed on Ficoll-Hypaque gradients and separated into B cell and T cell populations^{7,9}. Equal numbers of B cells (1×10^6) and T cells were incubated in culture with PWM ($50 \mu\text{g ml}^{-1}$, the concentration optimal for stimulating B and T cells⁷) in 5 ml of growth medium. After 7 d the culture supernatant fluids, free of cells, were analysed for IgG concentration by measuring the ability of various dilutions of supernatant fluid to block IgG-anti IgG binding. The reagents, their purity and the methodology for doing this assay were as before^{12,16}. The lower limit of immunoglobulin detection was 4 ng of IgG per ml. ND, none detected.

These results clearly show that T lymphocytes bearing Fc receptors for IgG (T_G) taken from the cord blood of human male newborns effectively suppress the division of their mothers' lymphocytes and lower the ability of B cells from the mother to make IgG *in vitro*. In contrast, the T-cell enriched lymphocyte population taken from human cord blood, then either depleted of T_G cells or bearing only IgM receptors (T_M) cannot suppress mitoses of maternal lymphocytes. These data are consistent with and extend the results of Moretta *et al.*, who showed that T_G lymphocytes obtained from adults dose-dependently suppressed

Table 3 T_G subpopulation of T lymphocytes from the human newborn suppresses production of immunoglobulin by lymphocytes of its mother

Source of lymphocytes		T cell population	IgG made (ng per 1 × 10 ⁶ B cells)	% Reduction
B cells	T cells			
Mother: BA	Mother: BA	Total	303	
Mother: BA	Newborn: BA	T _G	114	63
Mother: BA	Newborn: WE	T _G	108	65

Adult human peripheral blood or cord blood placed on Ficoll-Hypaque gradients was separated into B and T lymphocyte fractions. T cells from newborns were divided into distinct populations of T_G cells on the basis of rosetting with ox RBC. 1 × 10⁶ B cells from a mother were mixed with either 1 × 10⁶ total T cells from the same mother or 1 × 10⁶ T_G cells from her baby or an alien baby and cultured with PWM in 5 ml of RPMI growth medium. After 7 d the cultured supernatant fluids, free of cells, were analysed for IgG concentration (see Table 2 legend).

the differentiation and proliferation of B cells after stimulation with PWM in the presence of helper T_H cells⁹. No significant suppressor activity by peripheral blood lymphocytes collected from adults occurred unless T_G cells were first activated by interaction with immune complexes. In contrast, we found that T suppressor cells (T_G) in human cord blood are already active and able to suppress mitosis of maternal lymphocytes and production of immunoglobulin by maternal lymphocytes induced to differentiate with PWM. This indicates that lymphocytes from the baby are probably primed *in vivo* for their suppressor effect. Recently Maisson and his associates¹⁴ detected circulating antigen-antibody complexes in the sera of most pregnant women, suggesting a possible mechanism whereby T_G cells of a newborn may be activated. Further interaction of maternal and neonatal cells may generate T_G cells. Also suggestive of the biological importance of T_G cells in maternal-foetal interrelationships is the finding that three to fourfold more T_G cells are found in cord blood than in adults' blood (> 30% in the former compared with < 10% in the latter¹³).

How does this suppression work? Olding, Murgita and Wigzell using double culture chamber systems have shown that cord blood lymphocytes from a newborn effectively suppress the proliferation of lymphocytes from their mothers or other non-pregnant adults across a cell-semipermeable membrane, suggesting that inhibition of maternal lymphocyte proliferation occurs via a soluble suppressor factor(s)¹⁵. They also found that suppressor activity associated with newborn lymphocytes was completely abrogated by irradiation with 6,000 rad indicating the requirement for cell division in the *in vitro* release of suppressor factor. These findings are similar to those of Moretta *et al.*, who showed that treatment of activated T_G adult lymphocytes with 3,000 rad inhibited their ability to suppress polyclonal B cell differentiation and immunoglobulin production induced by PWM⁹.

Many factors in maternal-foetal interrelationships probably participate in preventing the automatic rejection of the foetus by its mother. Our studies suggest a mechanism by which foetal tissues can survive in a hostile immunological environment. We show that thymus derived suppressor lymphocytes, which are identified on the basis of bearing an Fc receptor for IgG, occur in infants' cord blood and perform an active process in inhibiting potentially harmful maternal lymphocytes from dividing. Further, the newborn's T suppressor cells are already primed in cord blood and after coming in contact with mothers' cells would render such cells unlikely to respond to the foetal foreign antigens. When this protective mechanism first develops is not clear, but necessarily it must arise after the foetal lymphoid system develops. The presence of foetal T_G cells late in pregnancy and their ability to release soluble suppressor factor(s) raise questions as to the level and fate of these cells during spontaneous abortions as well as the possible future use of suppressor factors as a therapeutic adjunct.

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- Faulk, W. P. & Temple, A. *Nature* **262**, 799-802 (1976).
- Goodfellow, P. N., Barnstable, C. J., Bodmer, W. F., Snary, D. & Crumpton, M. J. *Transplantation* (in the press).
- Jenkinson, E. J. & Billington, W. D. *Transplantation* **18**, 286-289 (1974).
- Currie, G. A., van Doorninck, W. & Bagshawe, K. D. *Nature* **219**, 191-192 (1968).
- Hellström, K., Hellström, I. & Braun, J. *Nature* **224**, 914-915 (1969).
- Olding, L. B. & Oldstone, M. B. A. *Nature* **249**, 161-162 (1974).
- Olding, L. B. & Oldstone, M. B. A. *J. Immun.* **116**, 682-686 (1976).
- Ferrarini, M., Moretta, L., Abrile, R. & Duranti, M. L. *Eur. J. Immun.* **5**, 70-72 (1975).
- Moretta, L., Webb, S. R., Grossi, C. E., Lydyard, P. M. & Cooper, M. D. *J. exp. Med.* (in the press).
- Olding, L. B., Benirschke, K. & Oldstone, M. B. A. *Clin. Immun. Immunopath.* **3**, 79-89 (1974).
- Moorhead, P. S., Nowell, P. C., Mellman, W. J., Battips, D. M. & Hungerford, D. A. *Exp. Cell Res.* **20**, 613-618 (1960).
- Waldmann, T. A. *et al.* *Lancet* **ii**, 609-613 (1974).
- Hayward, A. R. & Lawton, A. R. *J. Immun.* (in the press).
- Masson, P. L., Delire, M. & Cambiaso, C. L. *Nature* **266**, 542-543 (1977).
- Olding, L. B., Murgita, R. A. & Wigzell, H. *J. Immun.* (in the press).
- Lerner, R. A., McConahey, P. J., Jansen, I. & Dixon, F. J. *J. exp. Med.* **135**, 136-149 (1972).

Evidence for a common evolutionary origin of gastrin and cholecystokinin

THE gastrointestinal hormones gastrin and cholecystokinin (CCK) are structurally and functionally related in that they have identical COOH-terminal pentapeptide sequences, which represent the biologically active portions of the molecules. The rest of each molecule only modifies its activity quantitatively and determines relative potencies for various targets. This has suggested that the two hormones have evolved from a common ancestral molecule^{1,2}. To test this hypothesis we have investigated the occurrence of gastrin and CCK in different vertebrate species by immunocytochemistry and radioimmunochemistry. We now present evidence that in amphibians and teleosts gastrin and CCK are no longer recognisable as separate hormones.

To avoid problems with immunocytochemical cross-reactivity, antisera specific for the NH₂-terminal and middle sequence of gastrin were prepared. In submammalian species such antisera failed to demonstrate endocrine cells, although they were potent in demonstrating mammalian gastrin-producing cells. In contrast, antisera against the common COOH-terminal sequence of the two hormones reacted well with gastrointestinal endocrine cells of mammals, birds, reptiles, amphibians and bony fishes. This suggests that the biologically active COOH-terminal sequence is the only region of the gastrin molecule that has been preserved during evolution. Hence, submammalian gastrin cells can be demonstrated only with antisera that also react with the COOH-terminus of CCK. Therefore a specific CCK antiserum (no. 4698) that reacts with sequence 25-30 of porcine CCK-33, but fails to bind any gastrin (sulphated as well as non-sulphated) was used. Thus cells reactive with the COOH-terminus-specific antiserum but not with the CCK antiserum are called gastrin cells, whereas cells reactive with both antisera can be expected to contain a region similar to sequence 25-33 of CCK-33. In this way

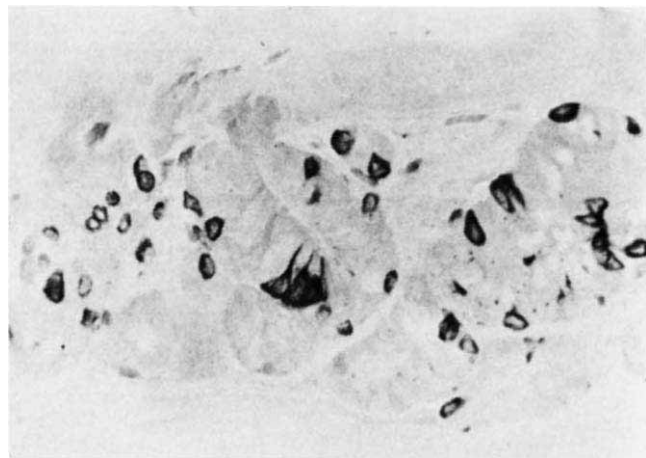


Fig. 1 Frog antral mucosa. Staining with CCK antiserum no. 4698 (PAP technique). Numerous immunoreactive epithelial cells are visible ($\times 280$). Specimens from gastrointestinal mucosa of mammals (man, pig and dog), birds (chicken and duck), reptiles (*Testudo graeca*), amphibians (*Rana esculenta* and *R. temporaria*) and bony fishes (*Gadus morrhua*) and from the brains of *R. esculenta* were frozen in melting Freon-22, freeze-dried, vapour-fixed with formaldehyde and embedded in paraffin. Human specimens were obtained at surgery (carcinoma) and animals were killed in slaughterhouses, by mebumal injections or by decapitation. Sections (3 μ m) were deparaffinised and subjected to indirect immunocytochemical staining for the demonstration of (1) NH₂-terminus of human gastrin-17 (antiserum no. 1295, given by Dr J. H. Walsh); (2) mid-sequence of human gastrin-17 (antiserum 4710); (3) COOH-terminus of human gastrin-17 (antisera nos 2604, 2609, 4562)^{13,14}; (4) sequence 25–30 of porcine CCK-33 (antiserum no. 4698); (5) middle region (probably sequence 19–25) of CCK-33 (antiserum no. 4478). Specificity of the antisera was ascertained using differential absorptions with: *a*, pentagastrin (Peptavlon, ICI); *b*, synthetic mid-sequence (6–13) of human gastrin-17 (given by Dr Morley); *c*, synthetic human gastrin-17 (SHG 1, ICI); *d*, highly purified porcine CCK-33 (given by Professor V. Mutt); *e*, synthetic caerulein (Caeruletide, given by Farmitalia, Milan). Absorptions were carried out at the optimal dilutions of the various antisera. Usually, addition of 10 μ g peptide per ml antiserum was sufficient. If the addition had no effect, the peptide concentrations were increased 10-fold. For details see refs 13 and 14. The site of antigen-antibody reaction was revealed with the peroxidase-anti-peroxidase (PAP) procedure of Sternberger¹⁵.

we ascertained that mammals (man, pig and dog), birds (chicken and duck) and a reptile (*Testudo graeca*) all contain distinct populations of gastrin and CCK cells. In these species, gastrin cells predominated in the antral mucosa whereas CCK cells occurred in the duodenum and jejunum. In amphibians (*Rana esculenta* and *R. temporaria*), however, no gastrin immunoreactive cells that did not also react with the specific CCK antiserum (no. 4698) were found (Fig. 1). CCK-immunoreactive cells predominated in the antrum but were also scattered in the small intestines. No CCK-immunoreactive cells that did not also react with the COOH-terminus-specific antisera were found. Absorption controls showed that, in all species, staining with the CCK antiserum (no. 4698) was abolished by pretreatment with CCK-33 or with caerulein, but was unaffected by pretreatment with even very large amounts of gastrin-17 (compare Fig. 1). Staining with the COOH-terminus-specific antisera was abolished by pretreatment with either pentagastrin, gastrin-17, CCK-33 or caerulein. Antiserum no. 4478, specific for the middle region (probably sequence 19–25) of CCK-33 and thus devoid of cross-reactivity to caerulein, stained no cells of the frog antral mucosa, whereas it was highly potent in demonstrating, for example, porcine CCK cells. Thus the compound present in the frog antral cells is dissimilar to CCK-33 in its middle region. Similar staining experiments with gastrointestinal mucosa of the teleost fish (*Gadus morrhua*) exactly reproduced the findings with amphibians, revealing a large population of

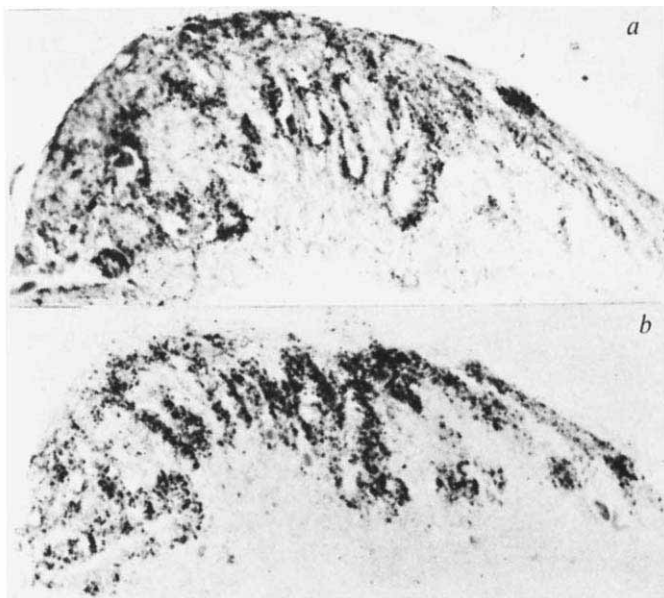
antral cells containing a region similar to sequence 25–33 of CCK-33. In the frog brain, many nerves showed the same pattern of immunoreactivity as the gastrointestinal endocrine cells. These nerves were most numerous in the hypothalamus where they converged towards the median eminence (Fig. 2).

In parallel with the immunocytochemical studies three individual extracts as well as pooled extracts of antrum and brain, respectively, from 30 frogs were subjected to gel chromatography as monitored by three assays: one specific for CCK and caerulein³, one measuring both CCK, caerulein and gastrin⁴ and one specific for gastrin⁴ (Fig. 3). As Fig. 3 shows, antral immunoreactivity eluted in three major peaks. The position of the peak of lowest apparent molecular size corresponded to that of caerulein. The intermediate peak eluted in a position corresponding to CCK-33 and gastrin-34 (big gastrin), whereas the size of the largest component corresponded to gastrin component I⁵ and a large CCK-component previously detected in extracts of porcine jejunum³. The immunoreactivity of the brain extracts also eluted in three major peaks in positions identical to those of the antral extracts. The smallest component, however, predominated in the brain extracts (Fig. 3). Gel chromatography of extracts of teleostean antrum and brain also revealed three immunoreactive peaks of which the quantitatively largest in the antral as well as in the brain extracts eluted in a position corresponding to caerulein.

Our data indicate that the three major components found in frog brain and antrum all contain a sequence similar to region 25–33 of CCK-33 and that the frog has no separate gastrin and CCK components. Gastrin immunoreactivity has been reported in the amphibian gastrointestinal tract⁶. Although no data concerning possible CCK immunoreactivity were given, the gel chromatographic data⁶ suggests that the peaks observed correspond to some of the components presented here.

The decapeptide caerulein and the nonapeptide phyllo-caerulein, both isolated from frog skin, have important gastrointestinal actions^{7,8}. In the frog, caerulein is a much more potent stimulator of gastric acid secretion than is gastrin-17 (ref. 9). Studies with synthetic analogues have

Fig. 2 Frog median eminence. *a*, Staining with CCK antiserum (no. 4698), PAP technique. Numerous dark-stained fibres and dots occur in the vicinity of vessels. *b*, Adjacent section to (*a*), for comparison stained with an antiserum against the growth hormone-release inhibiting hormone, somatostatin. The distribution of the somatostatin-containing nerves is similar but not identical to that of the CCK-immunoreactive nerves ($\times 256$).



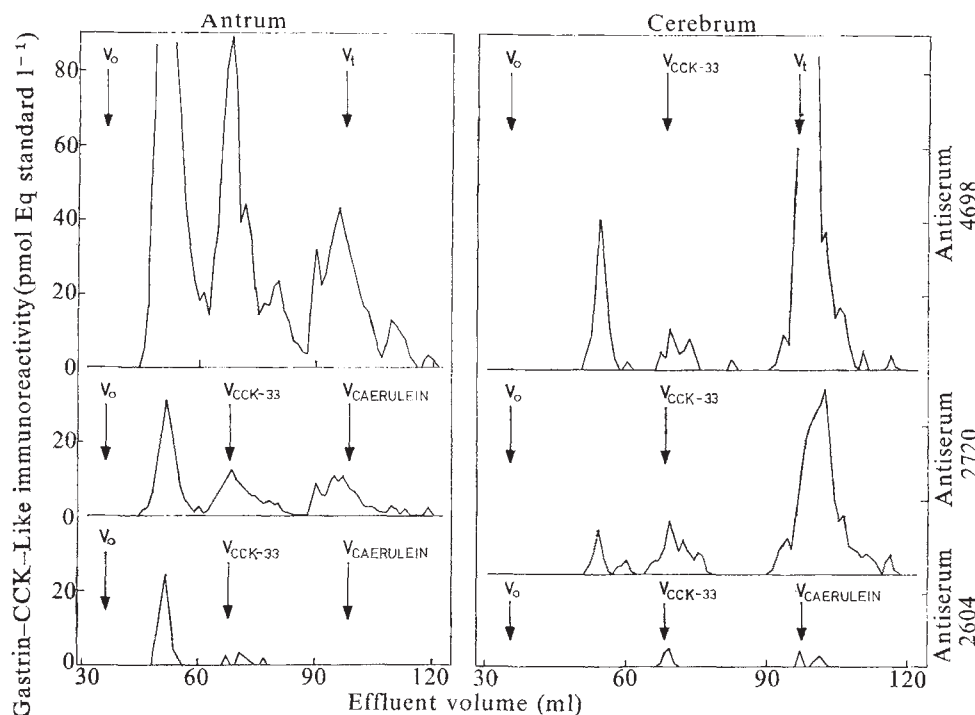


Fig. 3 Elution diagram of gastrin-CCK-like immunoreactivity in extracts of antrum and brain of the frog, *Rana esculenta*. Both individual antra and brains as well as pools of antrum and brains from 30 frogs were frozen immediately in liquid nitrogen and stored at -70°C . Brains and antra were minced while frozen and boiled for 30 min in water (10 ml per g tissue). After homogenisation, the immunoreactivity of the supernatant was measured by three assays. (1) The first was CCK-assay involving antiserum no. 4698 (raised against partially purified porcine (CCK-33, final dilution 1:20,000) and ^{125}I -CCK-33. This antiserum does not bind any of the known gastrins (sulphated or nonsulphated). With caerulein as standard, this assay measures 0.3 μg Eq per g cerebrum and 1.3 μg Eq per g antrum (wet weight). The second was an assay involving antiserum no. 2720 (ref. 4) (raised against synthetic human gastrin-17, final dilution 1:150,000) and ^{125}I -gastrin-17. The antiserum binds CCK-33 with a molar potency of 0.2 compared to gastrin-17. With synthetic human gastrin-17 as

standard, this assay measures 0.2 μg Eq per g cerebrum and 0.3 μg Eq per g antrum (wet weight). The third was a gastrin-assay involving antiserum no. 2604⁴ (raised against synthetic human gastrin-17, final dilution 1:200,000) and ^{125}I -gastrin-17. The antiserum binds CCK-33 with a potency of 0.002 compared to gastrin-17. With synthetic human gastrin-17 as standard, this assay measures 9.2 ng Eq per g cerebrum and 4.3 ng Eq per g antrum (wet weight). Antral and cerebral extracts were subsequently applied to Sephadex G-50 superfine columns ($11 \times 1000\text{mm}$) eluted at 4°C with 0.02M veronal, pH 8.4, containing 0.1% bovine serum albumin. The columns were eluted at a flow rate of 3.6 ml per h and fractions of 1.0 ml were collected. The immunoreactivity of each fraction was measured by the three assays. The columns were calibrated in separate gel filtrations with ^{125}I -albumin for indication of void volume (V_0), highly purified porcine CCK-33 ($V_{\text{CCK-33}}$), synthetic caerulein ($V_{\text{caerulein}}$) and $^{22}\text{NaCl}$ for indication of the total volume of the column (V_t). The extracts were also applied to Sephadex G-25 fine columns ($15 \times 90\text{mm}$) eluted at 4°C with 0.25 M NH_4HCO_3 , pH 8.2, calibrated with the same markers as the Sephadex G-50 columns. Fractions of 1.0 ml were measured by the three assays. The data are not shown in the figure because they agreed with results obtained on Sephadex G-50 columns. They revealed also three major peaks in both antral and cerebral extracts. The first peak appeared in the void volume, the second was eluted immediately after the void volume (K_{av} : 0.19) and the third was eluted after the salt peak (K_{av} : 1.21) in a position identical to that of caerulein. Extracts of pools from 15 antra and hypothalami of the teleost *Gadus morrhua* were treated exactly as frog brains and antra. Samples of 0.25 g teleost hypothalamus and 1.89 g teleost antrum were cut in small pieces in the frozen state and extracted as described here. Using the three assays, the following quantities of CCK-gastrin-like immunoreactivity were measured: (1) 108 ng Eq caerulein per g hypothalamus and 106 ng Eq caerulein per g antrum (Ab 4698); (2) 34.5 ng Eq gastrin-17 per g hypothalamus and 25.2 ng Eq gastrin-17 per g antrum (Ab 2720); (3) 1.7 ng Eq gastrin per g hypothalamus and 4.9 ng Eq gastrin-17 per g antrum (Ab 2604). By gel filtration on Sephadex G-50 superfine columns as described here, the immunoreactivity of both the cerebral and the antral extracts was eluted in three major peaks of which the quantitatively largest in both extracts occurred in a position corresponding to that of caerulein.

shown that in amphibians, in contrast to mammals, the occurrence of a sulphated tyrosyl-group in position 7 (as counted from the COOH-terminus), and not in position 6 as in gastrin (Table 1), is very important for acid stimulatory potency⁸. Thus, physiological evidence alone suggests that caerulein-like molecules are frog 'gastrins'. Our demonstration of a molecule with the size and immunological properties of caerulein in frog antrum tends to support this view. It is interesting that examination of the amino acid sequences of caerulein and phyllocaerulein shows that whereas these peptides resemble CCK in most of their sequence, they have similarities to gastrin in both their NH_2 - and COOH-terminal portions (Table 1). Thus, whereas in mammals, birds and reptiles CCK- and gastrin-like molecules occur in separate cell systems, the same cell seems to be responsible for the production of gastrin-CCK-like molecules in amphibians and bony fishes. Although comparisons with hormones of phylogenetic ancestors are impossible, the observations in frog as well as teleost antrum suggest that gastrin and CCK have evolved from a common caerulein-like ancestral molecule. Immunocytochemical studies have revealed cells reactive with antisera against caerulein as well as glucagon in cyclostomes¹⁰. Although the question whether the glucagon and caerulein-like immunoreactivity resides in a single molecule remains to be resolved, it is possible that the two main

families of gut hormones evolved from a single precursor occurring in very primitive vertebrates.

CCK-like peptides have been demonstrated in the brain of mammals^{11,12}. It is interesting that similar molecules exist in the brains of amphibians and fishes and that they have the same characteristics as the peptides found in the

Table 1 Amino acid sequence of caerulein, phyllocaerulein and the COOH-terminal decapeptides of porcine cholecystokinin and sulphated gastrin

	SO ₃ H
Cholecystokinin	-Asp-Arg-Asp-Tyr-Met-Gly-Trp-Met-Asp-Phe-NH ₂
	SO ₃ H
Caerulein	*Glu-Gln-Asp-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH ₂
	SO ₃ H
Phyllocaerulein	*Glu-Glu-Tyr-Thr-Gly-Trp-Met-Asp-Phe-NH ₂
	SO ₃ H
Gastrin	-Glu-Glu-Glu-Ala-Tyr-Gly-Trp-Met-Asp-Phe-NH ₂

*Glu, pyroglutamic acid.

gastrointestinal tract and skin. Immunocytochemical studies in frogs show that nerves containing these peptides are concentrated in the median eminence of the hypothalamus in much the same pattern as nerves containing known releasing or inhibiting factors to the pituitary (Fig. 2).

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- ¹ White, A., Handler, P. & Smith, E. L. *Principles of Biochemistry* fifth edn 916 (McGraw-Hill Kogakusha, Tokyo, 1973).
- ² Dockray, G. J. *Gastroenterology* 72, 344-358 (1977).
- ³ Rehfeld, J. F. *First. int. Symp. Gastrointestinal Hormones*, Asilomar, Abstr. no. 127 (1976).
- ⁴ Rehfeld, J. F., Stadil, F. & Rubin, B. *Scand. J. clin. Lab. Invest.* 30, 221-232 (1972).
- ⁵ Rehfeld, J. F. *Biochim. biophys. Acta* 285, 364-372 (1972).
- ⁶ Gibson, R. G., Mihás, A. A., Colvin, H. W. & Hirschowitz, B. I. *Proc. Soc. exp. Biol. Med.* 53, 284-288 (1976).
- ⁷ Anastasi, A., Erspamer, V. & Endean, R. *Arch. Biochem.* 125, 57-68 (1968).
- ⁸ Anastasi, A. *et al. Br. J. Pharmac.* 37, 198-206 (1969).
- ⁹ Negri, L. & Erspamer, V. *Naunyn-Schmiedeberg's Arch. Pharmac.* 277, 401-412 (1973).
- ¹⁰ Van Noorden, S. & Pearce, A. G. E. *Gen. comp. Endocr.* 23, 311-324 (1974).
- ¹¹ Vanderhaeghen, J. J., Signeau, J. C. & Gepts, V. *Nature* 257, 604-605 (1975).
- ¹² Dockray, G. J. *Nature* 264, 568-570 (1976).
- ¹³ Larsson, L.-I., Håkanson, R., Sjöberg, N.-O. & Sundler, F. *Gastroenterology* 68, 1152-1159 (1975).
- ¹⁴ Larsson, L.-I., Rehfeld, J. F., Håkanson, R. & Sundler, F. *Nature* 262, 609-610 (1976).
- ¹⁵ Sternberger, L. A. *Immunocytochemistry* (Prentice-Hall, Englewood Cliffs, New Jersey, 1974).

Gonadotrophin-releasing hormone deficiency in a mutant mouse with hypogonadism

FAMILIAL hypogonadism in man, due to an isolated deficiency of gonadotrophin secretion, has been well documented¹⁻⁶, but difficult to investigate because of the lack of a suitable animal model⁴. We report here the genetic and endocrinological background of a mutant strain of mouse in which the testes and ovaries fail to develop postnatally. The primary cause of this seems to be a deficiency in hypothalamic gonadotrophin-releasing hormone (GnRH) with a consequent reduction in pituitary content and circulating levels of luteinising hormone (LH) and follicle-stimulating hormone (FSH). By analogy with the Brattleboro rat (genetic defect in vasopressin synthesis) this mutant should prove useful for studying the synthesis of hypothalamic releasing hormones as well as the role of the hypothalamic-gonadotrophin system in sexual differentiation, puberty, folliculogenesis and spermatogenesis. The mutant has been named hypogonadal, symbol *hpg*.

The mutation was found at Harwell, segregating out from matings between three pairs of full sibs. Each of these was heterozygous for the Robertsonian translocation, Rb(1.3)1Bnr, which is of tobacco mouse (*Mus poschiavinus*) origin; and the matings represented the final step of establishing the translocation on a house mouse genetic background after 10 generations of backcrossing. The house mouse used throughout the crosses was the F₁ hybrid between two inbred strains, C3H/HeH and 101/H. Since the mutation has not been found in either inbred strain, or in the original mixed tobacco mouse/house mouse stock⁷ which carried the translocation, we have concluded that the mutation must have arisen in one of the animals of the latest backcross generations.

Breeding records generally indicated an autosomal recessive inheritance. Thus, *hpg* mice (both male and female) segregated out from matings that produced the mutant in accordance with the 25% expectation for this mode of inheritance (Table 1). In addition, all animals (both male and female) that were capable of producing *hpg* offspring in intercrosses, failed to do so when tested

in outcrosses to unrelated mice. When the progeny of such outcrosses were mated together, 7 out of 22 pairs again produced the mutant. This frequency is not significantly different from the 25% expected if *hpg* is inherited as an autosomal recessive. The only evidence discordant with this form of inheritance was the apparent low frequency of heterozygotes from matings that had been shown to be capable of producing the mutant. Two-thirds of the phenotypically normal progeny of such matings should be heterozygotes and, since they cannot be distinguished from their homozygous normal siblings, the expectation of two heterozygotes being mated together in random intercrosses should be 0.44. In fact, although at

Table 1 Segregation of *hpg* among 17 matings producing the mutant

Normal ♀♀ 219	Mutant ♀♀ 55	Progeny			
		NC ♀♀ 35	Normal ♂♂ 197	Mutant ♂♂ 69	NC ♂♂ 14

NC, Not classified for *hpg*; this includes only pre-weaning loss for males but both pre- and post-weaning loss for females.

least 20 offspring were checked per mating, only 10 out of 47 intercrosses of this origin were capable of producing *hpg* offspring. This is significantly different from the expectation. The reason for this discrepancy is not understood.

The segregation of the Robertsonian translocation in matings producing *hpg* has not been followed, but many of the animals used in the early crosses have proved to be chromosomally normal. There is therefore little possibility that *hpg* is linked to Rb(1.3)1Bnr, and this virtually rules out the proximal regions of chromosomes 1 and 3 as the location of *hpg* in the linkage map. No other mutants similar to *hpg* and, therefore possibly allelic, are yet known in the mouse.

Hypogonadal males can readily be distinguished from their normal male siblings by external examination. The penis is smaller than normal, the scrotum is undeveloped and the anogenital distance is much shorter than in normal males. Internally, all male reproductive organs are present

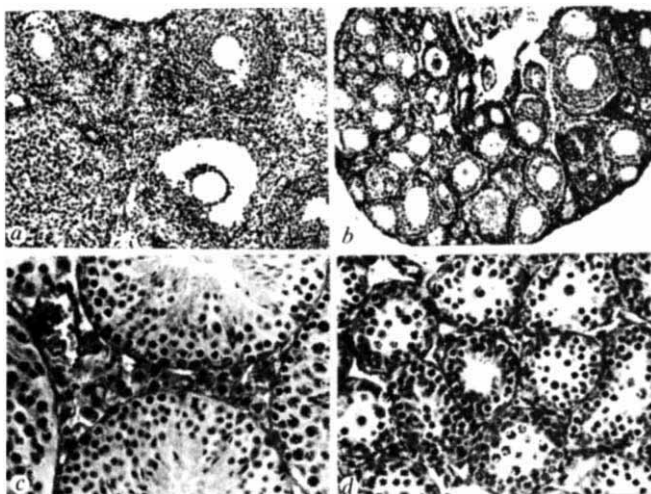


Fig. 1 *a*, Micrograph of ovary of 71-d-old normal female at same magnification as *b*. Well developed follicles and a corpus luteum can be seen. *b*, Micrograph of ovary of 71-d-old *hpg* female. None of the follicles shows any obvious signs of antrum formation. In most cases, the oocyte is surrounded by two layers of granulosa cells. *c*, Micrograph of testis of 71-d-old normal male at same magnification as in *d*. Here, spermatogenesis is complete, and the interstitial tissue is abundant and active. *d*, Micrograph of testis of 71-d-old *hpg* male. The failure of full development of spermatogenesis and the undeveloped state of the interstitial cells can be seen.

Table 2 Change of organ weight (mg) with age in normal and *hpg* mice of both sexes

Males	Age (d)	Testes		Seminal vesicles		Adrenal glands	
		Normal	Mutant	Normal	Mutant	Normal	Mutant
	31	110.7 ± 4.8	5.7 ± 0.4	12.4 ± 2.1	0.7 ± 0.1	3.5 ± 0.4	3.3 ± 0.4
	39	147.7 ± 16.9	—	48.4 ± 8.2	—	4.5 ± 1.2	—
	47	169.9 ± 0.7	4.9 ± 1.2	92.5 ± 6.6	1.0 ± 0.3	5.8 ± 0.5	4.2 ± 1.8
	65	158.7 ± 10.9	5.1 ± 0.4	152.4 ± 16.8	0.7 ± 0.2	4.5 ± 0.1	4.4 ± 1.7
	71	175.0 ± 3.1	4.3 ± 0.4	126.0 ± 42.3	1.4 ± 0.5	6.1 ± 1.0	4.2 ± 0.9
	89	187.6 ± 14.2	4.9 ± 0.5	182.9 ± 13.5	4.2(1)	4.0 ± 0.2	3.6 ± 0.4
	97	152.0 ± 22.3	5.6(2)	94.9 ± 28.7	1.8(2)	4.9 ± 0.4	5.3 ± 1.6
	112	169.9 ± 21.6	3.4 ± 1.3	132.0 ± 12.3	0.5 ± 0.3	5.6 ± 0.9	5.0 ± 0.9
Females	Age (d)	Ovaries		Uteri		Adrenal glands	
		Normal	Mutant	Normal	Mutant	Normal	Mutant
	31–42	4.5 ± 0.6	0.4 ± 0.1	42.3 ± 7.5	3.6 ± 0.7	3.7 ± 0.3	3.2 ± 0.5
	71	7.7 ± 2.4	0.2 ± 0.1	68.8 ± 23.0	3.5 ± 0.9	4.1 ± 1.0	5.2 ± 0.4
	88	8.5 ± 1.1	0.4 ± 0.2	107.0 ± 18.0	2.1 ± 0.4	3.1 ± 0.2	2.5(2)
	100–110	14.5 ± 2.6	0.5(2)	118.4 ± 14.9	2.9 ± 0.4	8.0 ± 0.7	6.5 ± 0.3

Means based on 3–10 animals per group, unless indicated by number in parentheses.

but immature. The testes, located in the abdomen, are very small.

The effect in females is similar. Externally, *hpg* females resemble normal females but can be distinguished with reasonable accuracy by the failure of the vagina to open fully. Internally, a thread-like uterus can be seen, and the ovaries are so small as to be distinguished only with difficulty.

Groups of three to ten normal and *hpg* mice of both sexes were killed at different ages, and body, gonadal, accessory sex organ and adrenal weights were recorded.

showed early stages of antrum formation (Fig. 1b). No luteal tissue was seen and interstitial tissue seemed to be atrophic.

Tables 3 and 4 show that the pituitary content of LH and FSH was markedly reduced in the mutant, although still assayable; the levels of hypothalamic GnRH were exceedingly low or undetectable. That the hypogonadotropism/hypogonadism is due primarily to a lesion involving GnRH synthesis was supported by the fact that plasma LH could be increased in *hpg* mice by injecting synthetic GnRH intravenously, but not by electrically

Table 3 Hypothalamic GnRH, pituitary and plasma gonadotrophic hormone levels during development in normal and *hpg* males

Age (d)	Hypothalamic GnRH (pg)		Pituitary LH (ng per pit.)		Pituitary FSH (ng per pit.)		Plasma FSH (ng per ml)	
	Normal	Mutant	Normal	Mutant	Normal	Mutant	Normal	Mutant
31	499 ± 264	7 ± 2	418 ± 110	25 ± 12	5,380(2)	389(2)	509 ± 159	54 ± 7
39	1,033 ± 330	—	892 ± 155	—	7,745 ± 1462	—	385 ± 92	—
47	1,446 ± 406	18 ± 3	1,304 ± 565	47 ± 9	13,512 ± 4696	218 ± 57	636 ± 355	65 ± 25
61–65	1,278 ± 303	5 ± 0.1	2,126 ± 237	67 ± 10	18,050 ± 1962	296 ± 44	656 ± 80	61 ± 5
71	1,668 ± 343	9 ± 2	1,983 ± 228	124 ± 22	18,286 ± 4273	313 ± 24	—	—
87	1,264 ± 313	10 ± 4	2,403 ± 46	74 ± 15	24,214 ± 2894	297 ± 48	478 ± 63	54 ± 3
97–99	1,814 ± 629	6 ± 1	1,337 ± 602	56 ± 22	18,051 ± 10285	199 ± 46	—	—
112–115	2,230 ± 251	12 ± 2	2,207 ± 549	109 ± 20	26,498 ± 6690	402 ± 76	—	—

GnRH was measured according to the procedures outlined in refs 10 and 11; gonadotrophins were measured according to the procedures outlined in refs 11–13. For each hormone, preliminary studies demonstrated parallelism between inhibition of binding curves produced by serial dilutions of the unknown and the appropriate standard. Standards were synthetic GnRH (ICI), NIH LH-S18, and NIAMD rat FSH-RPI. Means based on 3–10 animals per group unless indicated by number in parentheses.

Table 2 shows that the development of the testes, seminal vesicles, ovaries, and uteri of the normal mice contrasts with the total lack of change with age in the *hpg* animals.

Histological examination of the *hpg* testes revealed that in the majority of seminiferous tubules, spermatogenesis was rarely advanced beyond the diplotene stage. Interstitial tissue was scanty and appeared to be inactive (Fig. 1d). The ovaries contained mainly undeveloped follicles, and the majority of follicles which had undergone growth seemed to be arrested at the pre-antral phase; a few

stimulating the hypothalamus.

A number of parameters have been examined to determine whether other hypothalamo-hypophysial systems are affected in the *hpg* mutant. Body weights (g) of *hpg* females were similar to those of their normal female sibs either at 3 weeks of age (10.54 ± 0.13 , $n=19$ compared with 10.46 ± 0.66 , $n=63$) or at 6 weeks of age (19.51 ± 0.16 , $n=19$ compared with 19.80 ± 0.6 , $n=63$). Although *hpg* males were no smaller than their normal male sibs at 3 weeks of age (10.09 ± 0.16 , $n=26$ compared with

Table 4 Hypothalamic GnRH, pituitary and plasma gonadotrophic hormone levels during development in normal and *hpg* females

Age (d)	Hypothalamic GnRH (pg)		Pituitary LH (ng per pit.)		Pituitary FSH (ng per pit.)		Plasma FSH (ng per ml.)	
	Normal	Mutant	Normal	Mutant	Normal	Mutant	Normal	Mutant
31–36	1,207 ± 364	14(2)	596 ± 41	308(1)	2,207 ± 300	200(1)	—	—
41	831 ± 150	7 ± 2	591 ± 108	154 ± 51	1,098 ± 132	311 ± 89	—	—
71–72	831 ± 289	9 ± 5	514 ± 113	93 ± 5	1,040 ± 464	489 ± 107	211 ± 55	67 ± 6
83	623 ± 198	< 5	601 ± 236	136 ± 39	1,240 ± 358	548 ± 109	220 ± 89	64 ± 5
87–88	1,367 ± 518	9 ± 3	554 ± 129	215 ± 129	1,781 ± 284	687 ± 253	205 ± 34	51 ± 8
100	974 ± 440	4 ± 1	648 ± 163	73 ± 10	1,439 ± 213	378 ± 64	236 ± 87	60 ± 6
110	230 ± 42	9 ± 2	220(2)	—	779(2)	824(2)	—	—

Means based on 3–10 animals per group unless indicated by number in parentheses.

Table 5 Pituitary prolactin and ACTH in adult normal and *hpg* animals of both sexes

	Male		Female	
	Normal	Mutant	Normal	Mutant
Pituitary prolactin (ng/pit.)	141.6±16.0	54.7±9.7	269.8±89.3	92.3±6.6
Pituitary ACTH (ng/pit.)	147.4±24.3	73.6±14.2	94.0±8.7	87.4±4

Prolactin and ACTH were measured using the NIAMD rat assay kit and the method described in ref. 11, respectively. The standards were NIAMD rat prolactin-RP1 and III IWS ACTH (Mill Hill, London, UK). Means based on 5–8 animals per group.

9.85±0.12, *n*=51), by 6 weeks of age, however, they were significantly smaller (17.77±0.22, *n*=23 compared with 22.69±0.10, *n*=51), and their body weights more closely approximated those of their normal female siblings (19.80±0.06, *n*=63). This probably reflects a difference between the two groups in testosterone secretion, if it were due to a defect in growth hormone in the mutant, a difference between body weights would also be expected to occur in the female animals.

Histological studies of the thyroids from *hpg*, and normal mice showed no obvious differences in cell height of the follicular epithelium. This suggests that TSH production may be unaffected in the mutant.

As the foetal adrenal X zone is known to disappear at puberty in the male and during the first pregnancy in the female⁸, it is not surprising that histological examination indicated its presence in *hpg* males, and in both *hpg* and normal virgin females. As there was no significant difference in the width of the zona fasciculata/reticularis between mutant and normal males and females, adrenal function in the mutant would seem to be normal. There were, however, significant intergroup differences in pituitary adrenocorticotrophic hormone (ACTH) and prolactin content in the older animals (Table 5). Although we cannot exclude partial defects in the hypothalamo–ACTH and hypothalamo–prolactin axes, these differences probably reflect the effect which the hypothalamic–pituitary–gonadal system exerts on the other systems⁹.

Gross examination of the *hpg* mice has revealed no associated central nervous or cranio–facial abnormalities. This, together with the genetic findings, suggests that the mutant is akin to Ewer's series of human subjects³ who had familial monotrophic pituitary insufficiency transmitted as an autosomal recessive.

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- Kallman, F. J., Schoenfeld, W. A. & Barrera, S. E. *Am. J. ment. Defic.* **48**, 203–236 (1944).
- Nowakowski, H. & Lenz, W. *Rec. Progr. Horm. Res.* **17**, 53–89 (1961).
- Ewer, R. W. *J. clin. Endocr. Metab.* **28**, 783–788 (1968).
- Naftolin, F., Harris, G. W. & Bobrow, M. *Nature* **232**, 496–497 (1971).
- Santen, R. J. & Paulsen, C. A. *J. clin. Endocr. Metab.* **36**, 47–54 (1973).
- Boyar, R. M., Wu, R. H. K., Kapen, S., Hellman, L., Weitzman, E. D. & Finkelstein, J. W. *J. clin. Endocr. Metab.* **43**, 1268–1275 (1976).
- Cattanach, B. M., Williams, C. E. & Bailey, H. *Cytogenetics* **11**, 412–423 (1972).
- Chester-Jones, I. *Br. med. Bull.* **11**, 156–158 (1955).
- Brown-Grant, K. in *Regulation and Control in Living Systems* (ed. Kalmus, H.) 176–255 (Wiley, London, 1966).

- Nett, T. M., Akbar, A. M., Niswender, G. D., Hedlund, M. J. & White, W. F. *J. clin. Endocr. Metab.* **36**, 880–885 (1973).
- Chiappa, S. A. & Fink, G. J. *Endocr.* **72**, 195–210 (1977).
- Niswender, G. D., Midgley, A. R., Jr, Montec, S. E. & Reichert, L. E. Jr *Proc. Soc. exp. Biol. Med.* **128**, 807–811 (1968).
- Daane, T. A. & Parlow, A. F. *Endocrinology* **88**, 653–663 (1971).

Nigral and striatal dopamine release under sensory stimuli

SEVERAL groups have demonstrated the presence of a dopamine (DA)-sensitive adenylate cyclase in the substantia nigra. This adenylate cyclase is associated with postsynaptic dopaminergic receptors^{1–3}, supporting the hypothesis of a dendritic release of DA originally suggested by Björklund and Lindvall⁴. Other reports provided further arguments in favour of this hypothesis. Exogenous ³H-DA, taken up in slices of the rat substantia nigra, was shown to be released by potassium through a calcium-dependent process⁵. Furthermore, spontaneous and evoked release of ³H-DA has been demonstrated *in vivo* in the cat substantia nigra⁶. The increase of ³H-DA release in the substantia nigra induced by amphetamine (10^{–6} M) or benztropine (10^{–6} M) is associated with a reduction of the transmitter release from nerve terminals in the ipsilateral caudate nucleus, suggesting a decreased activity of the nigrostriatal dopaminergic⁷ neurones. A similar phenomenon was seen in the caudate nucleus when DA (10^{–7} M) was directly introduced into the substantia nigra⁸. We present here a study of the effects of unilateral sensory stimulations on the release of DA from nerve terminals and dendrites of the two nigrostriatal dopaminergic pathways. Previous studies⁹ revealed an increase in the levels of homovanillic acid in the perfusates of the cat lateral ventricle during electrical stimulation of the paws.

Groups of cats anaesthetised with halothane (2%) were implanted with four push–pull cannulae to simultaneously superfuse both caudate nuclei and both substantia nigrae using techniques detailed elsewhere^{10,11}. Purified L-3,5-³H-tyrosine (40 Ci mmol^{–1}), dissolved in an artificial cerebrospinal fluid (40 μCi ml^{–1}), was introduced in each push–pull cannula at a rate of 300 μl per 10 min using peristaltic pumps. ³H-DA released in successive 10-min fractions of superfusates was isolated from L-3,5-³H-tyrosine and ³H-metabolites by ion-exchange chromatography on Amberlite CG50 and the adsorption on alumina¹⁰. At steady-state level the quantities of ³H-DA released in the caudate nuclei and the substantia nigrae were 0.5 to 0.8 nCi per 10 min and 0.1–0.6 nCi per 10 min, respectively. They represented about 5–40 times the blank value which was identical to the counter background.

In a first experiment, a pair of electrodes was inserted into the paw of the right forelimb and a 10-min stimulation was carried out 3 h after onset of the superfusions using monophasic square pulses delivered by a Grass S 44 stimulator (4–6 V, 0.5 ms, 0.25 Hz) through a stimulus isolation unit. The intensity of the stimulus was adjusted in each experiment just below the threshold of the apparent muscle contraction. The 10-min stimulation of the paw of the right forelimb induced a significant activation (170%) of ³H-DA release in the contralateral substantia nigra when compared with the release of ³H-DA determined in corresponding fractions of control animals. This activation was associated

with a simultaneous reduction (30%) of the ^3H -transmitter release in the corresponding caudate nucleus which lasted for 1 h at least. An opposite pattern was observed in the side ipsilateral to the stimulation—the release of ^3H -DA was reduced in the substantia nigra and enhanced in the caudate nucleus (Fig. 1).

Similar results were obtained in a second experiment in which a visual sensory stimulation was substituted for the somatic electrical stimulation. In this case, the left eye was obturated and the right one was exposed for 10 min to a series of light flashes delivered with a frequency of 0.2 Hz. The two dopaminergic pathways reacted in opposite ways. In the side contralateral to the stimuli, the intense activation of ^3H -DA release in the substantia nigra was concomitant to the reduction of the ^3H -transmitter release from dopaminergic terminals and the reverse effects were seen in the ipsilateral side (Fig. 2).

The release of ^3H -DA into the substantia nigra can easily be modulated by physiological stimuli. As originally postulated⁴⁻⁶, it may originate from dopaminergic dendrites and not from recurrent axonal collaterals; the changes in the nigral release of ^3H -DA were always in the opposite direction to those observed in striatal terminals. This extends our previous data which indicated some differences in the characteristics of the release process of ^3H -DA in the substantia nigra and in the caudate nucleus⁵. Indeed, tetrodotoxin, a neurotoxin which blocks sodium channels, did not reduce the release of ^3H -DA in the substantia nigra in

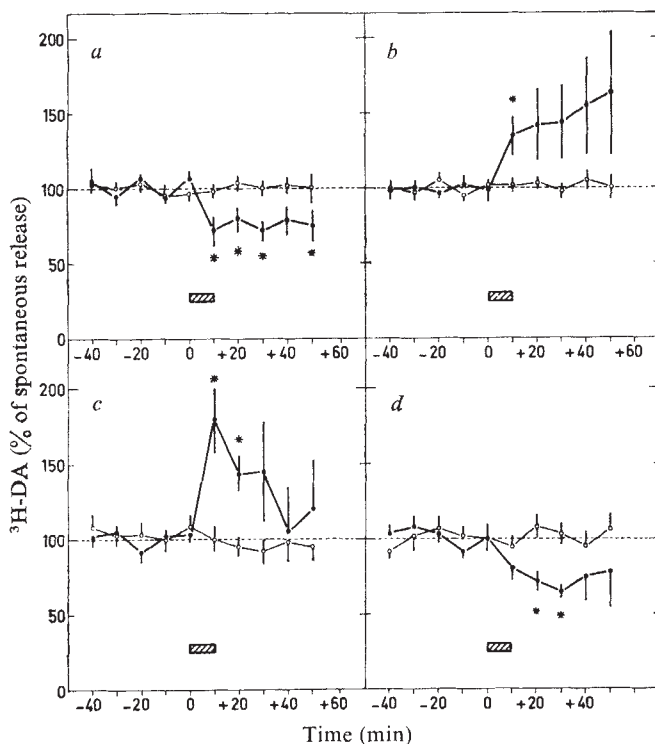


Fig. 1 Effects of unilateral somatic electrical stimulation on the release of ^3H -dopamine from the two caudate nuclei and the two substantia nigrae. Four push-pull cannulae were simultaneously implanted in the left (a) and the right (b) caudate nuclei and in the left (c) and the right (d) substantia nigrae. The four structures were perfused with an artificial cerebrospinal fluid containing L-3,5- ^3H -tyrosine. ^3H -dopamine was estimated in 10-min successive superfusate fractions. The paw of the right forelimb was stimulated (hatched bars) for 10 min using a pair of electrodes (monophasic square pulses, 4–6 V, 0.5 ms, 0.25 Hz). In each animal and for each cannula, ^3H -dopamine in each successive fraction was expressed as a percentage of an average spontaneous release calculated from the five fractions collected before the stimulation. Data are the mean $\pm$ s.e.m. of results obtained with five animals (a, b) or four animals (c, d). *, $P < 0.05$ when compared with corresponding control values ($\circ$) obtained in five non-stimulated animals.

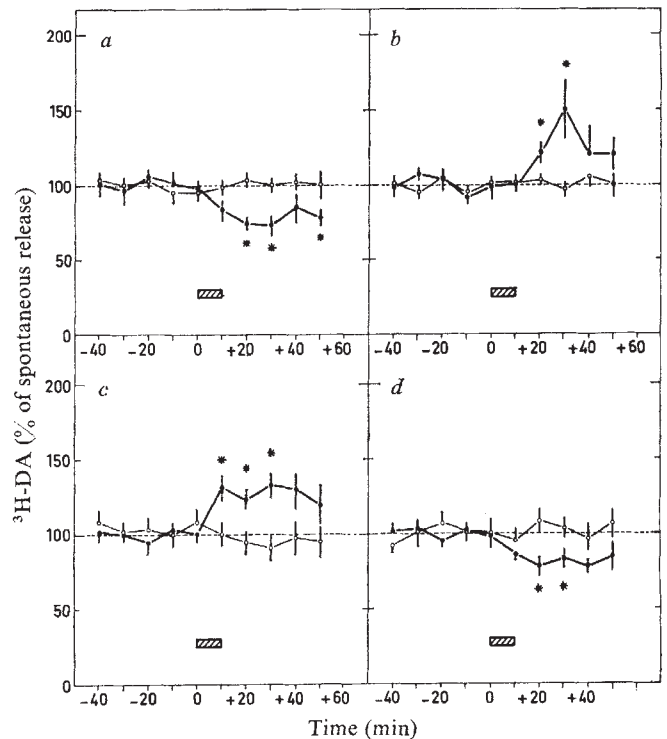


Fig. 2 Effects of unilateral visual stimulation on the release of ^3H -dopamine from the two caudate nuclei and the two substantia nigrae. Animals were treated as described in Fig. 1, except that the peripheral stimulation consisted of a series of light flashes (10 min, 0.2 Hz) directed to the right eye during the obturation of the left one. Data are calculated and expressed as in Fig. 1. *, $P < 0.05$ when compared with corresponding control values ($\circ$) obtained in five non-stimulated animals. a, Left, and b, right caudate nuclei. c, Left, and d, right substantia nigrae. Results for a–d are from five animals.

contrast to that observed in the caudate nucleus.

In the side contralateral to the somatic or visual stimulation, the activation of the dendritic release of ^3H -DA was always associated with a decreased activity of the dopaminergic neurones as indicated by the reduction of the transmitter release from nerve terminals. This phenomenon can be compared with the reduction of the activity of these neurones induced by the nigral application of DA^8 (10^{-7} M), amphetamine⁷ (10^{-6} M) or benzotropine⁷ (10^{-6} M), treatments which enhance the synaptic levels of the amine in the substantia nigra⁶. It may be related to the presence of autoreceptors on dopaminergic cells, first suggested on the basis of electrophysiological studies¹². Therefore in physiological states, it can be postulated that the activity of the dopaminergic neurones is inversely correlated to the extent of the dendritic release of the transmitter.

In a previous study¹³, we demonstrated the occurrence of a reciprocal control of the activity of the two nigrostriatal dopaminergic pathways. Briefly, treatments which selectively reduced the activity of one nigrostriatal dopaminergic system activated the release of DA from the nerve terminals of the contralateral dopaminergic neurones. This was observed shortly after the electrocoagulation of the left substantia nigra and during its superfusion with DA (10^{-7} M), amphetamine (10^{-6} M) or benzotropine (10^{-6} M). In these experimental conditions, the existence of opposite changes in the release of ^3H -DA from the nerve terminals of the two caudate nuclei was confirmed. If, as already discussed, the activity of the dopaminergic neurones is dependent on the extent of the dendritic release, it can be assumed that the opposite fluctuations in ^3H -DA release from nerve terminals are related to the reverse opposite

changes in the transmitter release occurring at the substantia nigra level.

In our initial demonstration of the reciprocal control of the activity of the two dopaminergic pathways¹³, the opposite changes in activity of these systems were triggered by physical or pharmacological manipulations of the dopaminergic neurones in one substantia nigra. Therefore, the marked opposite variations in the activity of the two systems seen under unilateral sensory stimulation could be initiated by inputs preferentially delivered on one pathway. But, nearly identical evoked potentials have been recorded in the two substantia nigrae during unilateral stimulation of the limbs¹⁴. Similarly, bilateral responses were also found in the caudate nuclei during unilateral somatic stimulation or unilateral visual stimulation¹⁵. The neuronal circuits involved in the opposite changes in the activity of the two dopaminergic pathways are not known, however.

Our results support the concept of the involvement of the nigrostriatal dopaminergic neurones in the integration of multisensory inputs¹⁶. In fact, unilateral degeneration of the ascending dopaminergic neurones is known to produce a pronounced deviation in movements and posture on the side ipsilateral to the lesion and a pronounced deficiency in the ability to orientate toward sensory stimuli which are presented to the contralateral side of the body¹⁶. The demonstration of the disymetric activity of the two dopaminergic pathways in response to unilateral sensory information should be of heuristic value for the understanding of the role of the extrapyramidal system in sensory motor integration.

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- 1 Gale, K., Guidotti, A. & Costa, E. *Science* **195**, 503-505 (1977).
- 2 Premont, J. et al. *FEBS Lett.* **68**, 99-104 (1976).
- 3 Kebabian, J. W. & Greengard, P. *Science* **174**, 1346-1349 (1971).
- 4 Björklund, A. & Lindvall, O. *Brain Res.* **83**, 531-537 (1975).
- 5 Geffen, L. B., Gessel, T. M., Cuello, A. C. & Iversen, L. L. *Nature* **260**, 258-260 (1976).
- 6 Nieoullon, A., Chéramy, A. & Glowinski, J. *Nature* **266**, 375-377 (1977).
- 7 Chéramy, A., Nieoullon, A. & Glowinski, J. *Interactions Among Putative Neurotransmitters in the Brain* (eds Garattini, S., Pujol, J. F. & Samanin, R.) (Raven, New York, in the press).
- 8 Chéramy, A., Nieoullon, A., Michelot, R. & Glowinski, J. *Neurosci. Lett.* **4**, 105-109 (1977).
- 9 Portig, P. J. & Vogt, M. J. *Physiol., Lond.* **204**, 687-715 (1969).
- 10 Nieoullon, A., Chéramy, A. & Glowinski, J. *J. Neurochem.* **28**, 918-928 (1977).
- 11 Chéramy, A., Nieoullon, A. & Glowinski, J. *Naunyn-Schmiedeberg's Arch. exp. Path. Pharmacol.* **297**, 31-37 (1977).
- 12 Bunney, B. S., Aghajanian, G. K. & Roth, R. H. *Nature new Biol.* **245**, 123-125 (1973).
- 13 Nieoullon, A., Chéramy, A. & Glowinski, J. *Science* (in the press).
- 14 Féger, J., Ohye, C., Jacquemin, J. & Martin, A. J. *Physiol., Paris* **69**, 247 A (1974).
- 15 Albe-Fessard, D., Oswald-Cruz, E. & Rocha-Miranda, C. *Electroencephal. clin. Neurophysiol.* **12**, 405-420 (1966).
- 16 Ljungberg, T. & Ungerstedt, U. *Exp. Neurol.* **53**, 585-600 (1976).

In addition, chlordiazepoxide mimics GABA in decreasing the firing rate of rat brain neurones⁶. These, and other, actions of benzodiazepines are blocked by the GABA antagonists, picrotoxin and/or bicuculline. Precise interpretation of such indirect evidence is difficult in view of the complexity of the intact brain, and the picture is far from clear. One physiologic study has concluded that benzodiazepines are potent GABA antagonists⁷. Another study based on benzodiazepine displacement of ³H-strychnine binding to CNS membranes suggested that these drugs may interact with glycine receptors rather than GABA receptors⁸. We have analysed the effect of chlordiazepoxide (CDPX) on GABA and glycine responses in spinal cord cell cultures, where individual neurones can be visualised and tested directly. Our results suggest that CDPX selectively facilitates the action of GABA, whether exogenously applied or synaptically released, by a direct action on the postsynaptic membrane.

Cells were dissociated from 4-7-d embryonic chick spinal cords, and plated in collagen-coated 35-mm tissue culture dishes, as previously described⁹. Electrophysiologic experiments were performed on the stage of an inverted microscope equipped with phase-contrast optics. The cells were perfused with oxygenated nutrient medium and temperature (34-36 °C) and pH (7.4) were carefully controlled. Individual neurones were penetrated for intracellular recording with 4 M potassium acetate or 3 M potassium chloride filled microelectrodes ($R = 50-100$ M Ω). Membrane conductance was monitored by injecting pulses of inward current through the recording electrode (connected in a bridge circuit). Drugs were applied by pressure ejection from blunt tip (3-5 μ m) pipettes; this technique allows application of known drug concentrations. GABA and glycine were also applied by iontophoresis from fine tip (< 0.5 μ m) double barrel pipettes.

Recordings were taken from over 200 cells studied 11-40 d after plating. The cell bodies of neurones selected ranged between approximately 20 μ m and 50 μ m in diameter. All neurones tested were sensitive to GABA and glycine. Both drugs were applied sequentially to 38 cells. With a potassium acetate electrode, and a good penetration (resting potential -70 mV to -80 mV), application of either drug produced a small depolarising response associated with a marked increase in membrane conductance.

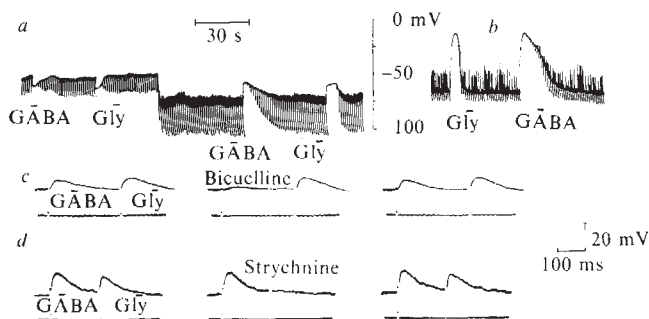


Fig. 1 GABA and glycine (Gly) responses. *a* and *b* are slow-speed tracer records of responses to pressure ejected GABA (1 mM) and glycine (1 mM). Horizontal lines below the records indicate the pressure pulse duration. Vertical lines are electronic potentials produced by inward current pulses (1 nA for 100 ms in *a*, 0.26 nA for 100 ms in *b*): their length is proportional to the cell input resistance. In (*a*) the cell was penetrated with a potassium acetate electrode, and GABA and glycine responses were evoked at two membrane potentials (note the vertical scale). Both responses reversed polarity at about -60 mV. *b* shows responses recorded in another cell with a potassium chloride electrode. After Cl⁻ loading, both drugs depolarised the cell to about -10 mV. Baseline irregularity in these and other records reflect spontaneous synaptic activity. *c* and *d* are oscilloscope traces showing fast GABA and glycine responses (potassium chloride recording electrode) following iontophoresis from a double barrel pipette. Iontophoretic current pulses are shown below each trace. The paired responses were evoked before (left), during (middle) and 1 min after (right) pressure ejection of the indicated antagonists. The voltage calibration bar also indicates 0.4 μ A in *c* and 1.0 μ A in *d*. Bicuculline was added at 2.2×10^{-4} M and strychnine at 10^{-5} M.

Chlordiazepoxide selectively augments GABA action in spinal cord cell cultures

THE clinical actions of benzodiazepines—relief of anxiety, suppression of convulsions, and relaxation of skeletal muscle—suggest that they enhance inhibition in the central nervous system (CNS) but their mechanism of action is unknown. Indirect evidence^{1,2} indicates that benzodiazepines augment the action of γ aminobutyric acid (GABA), a putative inhibitory transmitter. For example, diazepam increases presynaptic inhibition in the spinal cord^{3,4}, a type of inhibition that may reflect GABA action on primary afferent terminals⁵.

These voltage responses reversed direction when the cell was depolarised above -50 mV to -60 mV (Fig. 1a). As expected from work in other systems (for review see ref. 10), the increase in membrane conductance induced by GABA or glycine reflects primarily an increase in permeability to Cl^- ions. When KCl filled recording electrodes were used, the reversal potential for both drugs was shifted towards 0 mV (Fig. 1b). Pressure ejection of bicuculline or strychnine attenuated GABA and glycine responses, respectively (Fig. 1c, d).

Pressure ejection of CDPX immediately produced a striking potentiation of the GABA response, while having no effect on the glycine response (Fig. 2a). The potentiation was rapidly reversible after termination of the CDPX pulse, but it was maintained for

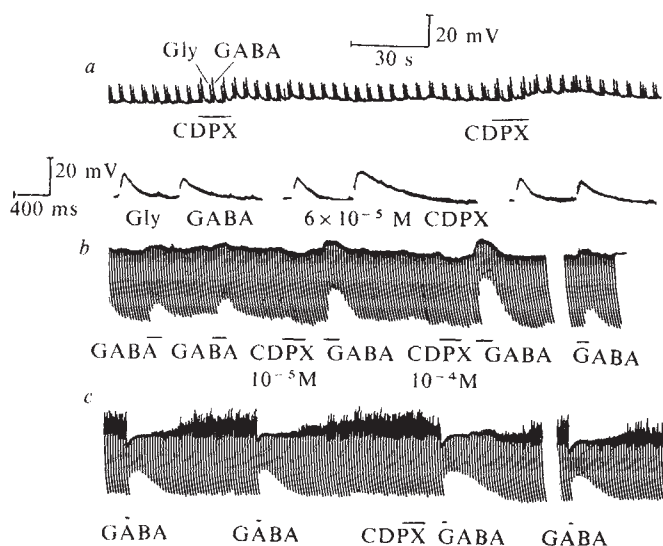


Fig. 2 CDPX potentiation of GABA responses. In *a*, paired iontophoretic glycine and GABA responses are shown on a slow speed tracer (top). Note the selective potentiation of the GABA potential. The same phenomenon is shown in fast oscilloscope sweeps (below) taken before, during and after pressure ejection of CDPX ($60 \mu\text{M}$). Note that different sets of calibration bars apply to top and bottom records. The late depolarisation seen here subsequent to CDPX application (top) probably reflects potentiation of GABA that leaks from the closely applied iontophoretic pipette. It was never observed in the absence of a GABA pipette. *b*, A trace illustrating that potentiation of submaximal GABA responses depends on CDPX concentration. $6 \mu\text{M}$ GABA was applied by pressure ejection at the indicated times. The small hyperpolarisation coincident with CDPX ejection is an artefact. Spontaneous synaptic potentials were suppressed in this experiment with tetrodotoxin ($3.4 \times 10^{-8} \text{ M}$). The break in the record represents a 2-min interval. *c*, Trace illustrating that a maximum (1 mM) GABA response is prolonged by CDPX (10^{-4} M) but the peak potential change and conductance increase are unchanged. Break in baseline, 4 min. Inward current pulses, 0.6 nA for 100 ms in *b*; 0.3 nA for 100 ms in *c*. Calibration bars in *a* (top) apply also to *b* and *c*. Potassium chloride recording electrode was used in *a*; potassium acetate in *b* and *c*.

several hours in the continued presence of CDPX. CDPX alone, at potentiating concentrations (10^{-7} M to 10^{-4} M), had no effect on the resting membrane potential or membrane conductance. Potentiation of GABA responses increased with increasing CDPX concentration between 10^{-7} M and 10^{-4} M (Fig. 2b), with an ED_{50} (50% effective concentration) of approximately 5 – $10 \mu\text{M}$ (44 cells). (The conductance caused by GABA was calculated assuming a shunt was inserted in parallel with the resting membrane conductance, and the CDPX potentiation was expressed as a percentage increase in this specific GABA conductance.) The CDPX pulse preceded the GABA pulse (Fig. 2b), so the effective CDPX concentration probably is lower than the pulse concentration, and these estimates of the ED_{50} must be considered upper limits. CDPX increased the magnitude of submaximal GABA responses.

The response to a maximal dose of GABA (1 mM) was prolonged, but the peak response was unchanged (13 cells) (Fig. 2c).

CDPX potentiation of GABA chemosensitivity might be due to: (1) blockade of GABA uptake; (2) induction of GABA release; or (3) a direct effect on the postsynaptic membrane. Blockade of uptake is unlikely. Our transport studies failed to demonstrate an effect of 1 mM CDPX on ^3H -GABA uptake in sister cultures. Also, 1 mM L-2,4-diaminobutyric acid, a known inhibitor of neuronal GABA uptake¹¹, did not alter the potentiating effect of CDPX (five cells). Induction of GABA release cannot account for the potentiation of large GABA responses in the absence of a GABA-mimetic effect. A direct postsynaptic effect on GABA receptors is most likely. Since CDPX at potentiating concentrations is not an agonist, it is probably not acting at the GABA binding site. It may bind to a regulatory site on or near the GABA receptor, and alter either the affinity of the receptor for GABA, or the coupling between GABA binding and opening of membrane chloride channels. This conclusion is consistent with a recent description of saturable, high affinity binding of ^3H -diazepam in rat brain¹². The ^3H -diazepam binding could not be inhibited by GABA or GABA antagonists.

In addition to potentiation of exogenously applied GABA, CDPX augments synaptic potentials that are probably mediated by GABA. Synaptic potentials were evoked by extracellular stimulation of nearby neurones or nerve processes, and KCl recording electrodes were used to convert small inhibitory potentials into large depolarising responses. Large Cl^- dependent synaptic potentials that were attenuated by bicuculline were augmented by CDPX (six cells) (Fig. 3). As expected of a drug which increased GABA-ergic inhibition, CDPX often reduced the overall frequency of spontaneous synaptic potentials.

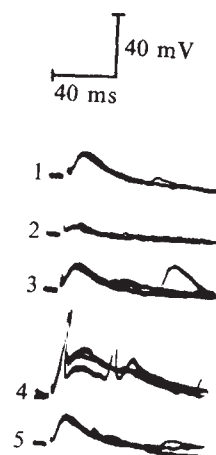


Fig. 3 CDPX potentiation of a bicuculline sensitive synaptic potential. The stimulus evoked synaptic potentials labelled 1–5 were recorded with a potassium chloride electrode and were obtained in sequence. 1, Control; 2, during ejection of 10^{-4} M bicuculline; 3, return to control 1 min later; 4, during ejection of $1.2 \times 10^{-4} \text{ M}$ CDPX; 5, return to control. The stimulus artefact appears as a break in the trace immediately before the evoked response. Spontaneous synaptic potentials appear late in the records. Each record is the superposition of three consecutive oscilloscope sweeps.

This demonstration of potentiation of GABA-ergic synaptic potentials as well as of exogenously applied GABA supports the hypothesis that some central actions of benzodiazepines are associated with enhanced GABA-ergic inhibition. We found no evidence for interaction with glycine receptors. It should be noted that the CDPX ED_{50} for GABA potentiation (5 – $10 \mu\text{M}$) is at least 20 times smaller than the ED_{50} reported⁸ for ^3H -strychnine displacement ($200 \mu\text{M}$). In any case, the selectivity of CDPX, as a complement to the GABA antagonists, should prove useful in identifying and characterising GABA-ergic pathways in the CNS.

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- ¹ Costa, E., Guidotti, A. & Mao, C. C. in *GABA in Nervous System Function* (eds Roberts, E., Chase, T. N. & Tower, D. B.) 413-426. (Raven, New York, 1976).
- ² Haefely, W. et al. in *Mechanism of Action of Benzodiazepines* (eds Costa, E. & Greengard, P.) 131-151. (Raven, New York, 1975).
- ³ Schmidt, R. F. *Ergebn. Physiol.* **63**, 20-101 (1971).
- ⁴ Stratten, W. P. & Barnes, C. D. *Neuropharmacology*, **10**, 685-696 (1971).
- ⁵ Barker, J. L. & Nicoll, R. *Science*, **176**, 1043-1045 (1972).
- ⁶ Dray, A. & Straughan, D. W. *J. Pharm. Pharmacol.*, **28**, 314-315 (1976).
- ⁷ Gahwiler, B. H. *Brain Res.*, **107**, 176-179 (1976).
- ⁸ Young, A. B., Zukin, S. R., Snyder, S. H. *Proc. natn. Acad. Sci. U.S.A.*, **71**, 2246-2250 (1974).
- ⁹ Fischbach, G. D. & Dichter, M. A. *Dev. Biol.*, **37**, 100-116 (1974).
- ¹⁰ Krnjević, K. *Physiol. Rev.*, **54**, 418-540 (1974).
- ¹¹ Iversen, L. L. & Johnston, G. A. R. *J. Neurochem.*, **18**, 1939-1950 (1971).
- ¹² Squires, R. F. & Braestrup, C. *Nature*, **266**, 732-734 (1977).

Phosphorylation of chloroplast membrane polypeptides

ILLUMINATION of chloroplast thylakoids leads to the formation of the so-called high energy state of the membrane¹⁻³. The establishment of this state is accompanied by several structural changes within the membrane, including a conformational change in the coupling factor⁴, increased accessibility of photosystem II to the chemical probe *p*-diazonium benzene sulphonate⁵, and a reduction in the thickness of the partition between stacked thylakoids⁶. I describe here a rather different type of structural change that has not previously been reported for chloroplast membranes—protein phosphorylation. Like the above changes, protein phosphorylation is a reversible, energy-dependent membrane modification, but it differs from the other changes in that it takes the form of a specific chemical reaction involving certain identifiable chloroplast membrane polypeptides. The most conspicuous of these polypeptides is the light-harvesting chlorophyll *a/b* binding protein, the most abundant thylakoid polypeptide⁷.

Isolated class A or class B (ref. 8) chloroplasts of the pea (*Pisum sativum* L. var. Feltham First) incorporated ³²P-orthophosphate into ATP, CTP, GTP, UTP and RNA in the light⁹. In identical conditions of chloroplast isolation and incubation, protein phosphorylation is also readily demonstrated. After a standard incubation period of 10 min, labelled chloroplasts were washed, solubilised in boiling sodium dodecyl sulphate (SDS) solution, and fractionated by SDS-polyacrylamide slab gel electrophoresis¹⁰. Chloroplast proteins were detected by staining the gel with Coomassie Brilliant Blue (Fig. 1a) and radioactivity was located by autoradiography (Fig. 1b). Many polypeptides were visible after staining but three were particularly prominent: (1) the 54,000 molecular weight (MW) large subunit of ribulose 1,5-bis-phosphate (RBP) carboxylase, the most abundant soluble chloroplast protein¹¹, (2) the 12,000 MW subunit of the same enzyme, and (3) the 26,000 MW subunit of the light harvesting chlorophyll *a/b* binding protein complex⁷. There were two major phosphorylated polypeptides: (1) a 26,000 MW polypeptide which co-migrated in every experiment with the chlorophyll *a/b* binding protein, and (2) a 9,000 MW polypeptide whose MW was estimated by comparison with cyanogen bromide cleavage fragments derived from cytochrome *c*¹². Other phosphorylated polypeptides were also evident, their molecular weights falling generally in the

the range 7,000–70,000. Neither subunit of RBP carboxylase was labelled *in vitro*.

In many experiments, it was possible to resolve the 26,000 MW polypeptide into two labelled species (Fig. 2). The MW of the second polypeptide was estimated to be 28,000. These two polypeptides, together with the 9,000 MW polypeptide, were eluted from the gel¹³ and subjected to brief acid hydrolysis. The digestion products were separated by paper electrophoresis and shown to be phosphothreonine and orthophosphate. During acid hydrolysis phosphothreonine breaks down to threonine and orthophosphate. It is therefore likely that the 65% recovery of isotope in phosphothreonine underestimates the percentage of the label in this form in the native protein. It is possible, however, that some of the labelled orthophosphate was derived from other less stable phosphorylated amino acids such as phosphohistidine. At present, we can only say that most of the ³²P-orthophosphate incorporated into the 28,000, 26,000 and 9,000 MW polypeptides was recovered in phosphothreonine.

The 26,000 and 9,000 MW polypeptides were found to be membrane-bound and to be labelled *in vivo* (Fig. 3). The recovery of all but the 70,000 MW polypeptide in the thylakoid and envelope fraction of osmotically shocked chloroplasts indicates that chloroplast protein phosphorylation is essentially a membrane phenomenon and supports the conclusion reached earlier that RBP carboxylase was not phosphorylated *in vitro*.

Leaf pairs were allowed to take up ³²P-orthophosphate

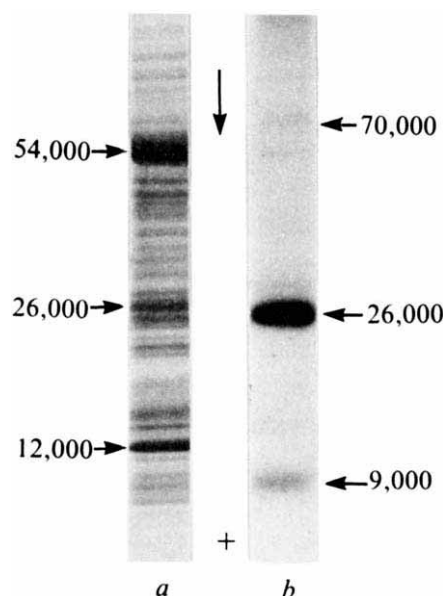


Fig. 1 *In vitro* incorporation of ³²P-orthophosphate into chloroplast phosphoproteins. Chloroplasts from 10-d-old pea seedlings were isolated and resuspended in isotonic KCl medium as described previously^{8,15}. An aliquot (100 µg chlorophyll in 0.5 ml) was incubated at 20 °C for 10 min with carrier-free ³²P-orthophosphate (100 µCi). Illumination was by a single coil 200-W tungsten lamp (Philips). After incubation the chloroplasts were diluted with 10 ml of ice-cold 0.35 M sucrose medium containing 25 mM HEPES-NaOH buffer (pH 7.6), 2 mM EDTA and 2 mM sodium isoascorbate, and centrifuged at 2,800g for 2 min. The chloroplast pellet was washed twice in acetone-water (4:1) and then taken up in 400 µl of 2% SDS solution containing 60 mM Tris-HCl buffer (pH 6.8), 0.5% 2-mercaptoethanol and 10% glycerol. After 3 min at 100 °C, the solution was cooled and an aliquot (40 µl) was electrophoresed through a 10%–30% exponential gradient slab gel of acrylamide which included a 5% stacking gel¹⁰. The gel was stained with Coomassie Brilliant Blue, photographed and autoradiographed. *a*, Photograph of stained gel showing total chloroplast protein, *b* Autoradiogram showing chloroplast phosphoproteins. Molecular weights were estimated by comparison with known standards, including cyanogen bromide cleavage fragments of cytochrome *c*¹².

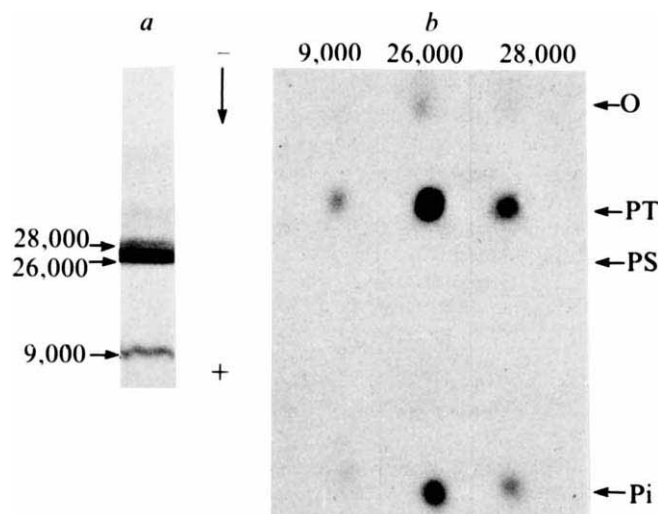


Fig. 2 Identification of threonine as site of phosphorylation in three chloroplast phosphoproteins. *a*, Chloroplast phosphoproteins were labelled *in vitro* for 10 min, fractionated electrophoretically and located by autoradiography. *b*, Bands containing the 28,000, 26,000 and 9,000 phosphoproteins were cut from the gel slab and the polypeptides were extracted as tryptic peptides¹³. After lyophilisation the tryptic peptides were digested under N₂ in 6 M HCl (105 °C, 90 min) and analysed by electrophoresis on Whatman 3 MM paper using formic acid-acetic acid-water (1:2:45) buffer (pH 2.0). Autoradiography showed that radioactive material was located at the origin (O) and also found in phosphothreonine (PT) and orthophosphate (Pi) but not in phosphoserine (PS).

for 6 h under constant illumination and were then homogenised and chloroplasts were isolated. Electrophoresis of the solubilised polypeptides showed that the 26,000 and 9,000 MW polypeptides were again the most heavily labelled chloroplast phosphoproteins and therefore that the *in vitro* labelling of these polypeptides was not an artefact of chloroplast isolation but represented a physiological process. The broad band (X) labelled *in vivo* was phospholipid.

The *in vitro* phosphorylation of chloroplast membrane

Table 1 *In vitro* phosphorylation and dephosphorylation of chlorophyll *a/b* binding protein within isolated chloroplasts

Incubation conditions		³² P-incorporation (10 min light – 100)
Light	0 min	1
	1 min	12
	5 min	48
	10 min	100
	20 min	88
	30 min	72
	10 min + 20 µM CCCP	1
Dark	10 min	1
Light (10 min) followed by	10 min light	88
	10 min dark	9
	10 min light + 20 µM CCCP	20
	10 min light + 1 mM KH ₂ PO ₄ (pH 8.3)	37

Replicate aliquots of a suspension of pea chloroplasts (100 µg chlorophyll in 0.5 ml isotonic KCl medium) were pre-incubated for 10 min in the dark at 20 °C with or without 20 µM CCCP. Radioisotope (100 µCi ³²P-orthophosphate) was then added to each replicate and incubation was continued in the light or the dark as indicated. After the incubations the chloroplasts were washed, solubilised and fractionated electrophoretically as described in Fig. 1. After the position of the chlorophyll *a/b* binding protein (26,000 MW polypeptide) had been determined by staining and autoradiography, the band of gel containing it was excised from each track and ³²P was determined by measuring Cerenkov radiation in a liquid scintillation spectrometer (counting efficiency 45%). Incorporation into the chlorophyll *a/b* binding protein is expressed relative to the incorporation into this protein after 10 min illumination (1.5 × 10⁴ d.p.m. per gel slice, or 1.5 × 10⁶ d.p.m. per incubation).

polypeptides was not linear with time (Table 1). Incorporation of ³²P into the 26,000 MW polypeptide was rapid for 5–10 min and thereafter was reversed. The loss of radioactivity from the 26,000 MW band was greater than could be explained by chloroplast disintegration during incubation or washing. Two processes determined the instantaneous level of protein labelling—phosphorylation and dephosphorylation. Protein phosphorylation was driven by light through photophosphorylation. In this respect it resembles RNA and protein synthesis in isolated pea chloroplasts^{14,15}. Dephosphorylation was readily demonstrated by incubating chloroplasts for 10 min in the light to label the 26,000 MW polypeptide and then subjecting the chloroplasts to various treatments to inhibit or chase protein phosphorylation: (1) dark incubation, (2) illumination in the presence of the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP), and (3) a chase with 1 mM unlabelled orthophosphate. In each case, rapid loss of radioactivity from the chloroplast proteins was observed. Thus, chloroplast protein phosphorylation is reversible, being sensitive to the ATP levels within the organelle.

Chloroplast protein phosphorylation has not previously been reported, although protein kinase activity has been

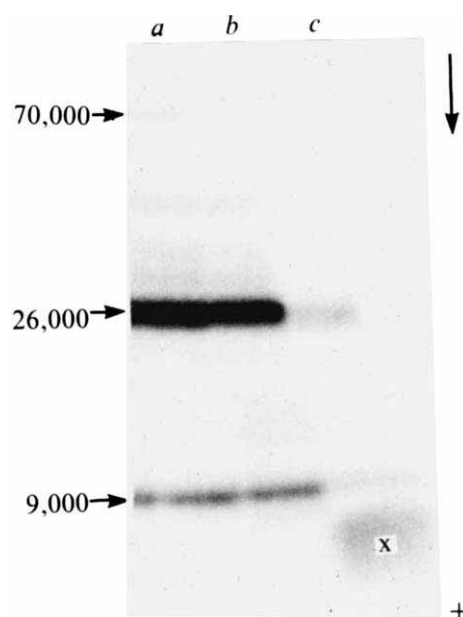


Fig. 3 Location and *in vivo* labelling of chloroplast phosphoproteins. Isolated chloroplasts were incubated *in vitro* with ³²P-orthophosphate for 10 min and then washed in ice-cold sucrose medium. Half of the chloroplasts were solubilised immediately in SDS solution and the remainder were resuspended in hypotonic medium (20 mM Tris-HCl, pH 8.0, 4 mM MgCl₂, 2 mM 2-mercaptoethanol) and centrifuged at 30,000g for 30 min. The pellet, containing thylakoids and envelopes, was solubilised in SDS solution. Chloroplasts were also isolated from 10 leaf pairs which had taken up ³²P-orthophosphate (50 µCi per leaf pair) for 6 h under continuous illumination. The radioactive chloroplast pellet was solubilised in SDS solution and electrophoresed alongside the two *in vitro* labelled samples. *a*, Total chloroplast proteins, labelled *in vitro*. *b*, Total chloroplast membrane proteins, labelled *in vitro*. *c*, Total chloroplast proteins labelled *in vivo*. The broad band (X) in track *c* represents labelling in phospholipid.

attributed to chloroplasts from *Acetabularia*¹⁶ and to a fraction from *Brassica* containing nuclei and chloroplasts¹⁷. I have shown that two chloroplast membrane polypeptides (with MWs of 26,000 and 9,000) are particularly heavily phosphorylated *in vivo* and *in vitro*, with several other polypeptides also being phosphorylated to a detectable extent *in vitro*. The polypeptide of 26,000 MW co-electrophoreses with the light-harvesting chlorophyll *a/b* binding protein, the most abundant thylakoid polypeptide.

Other evidence that these two polypeptides are identical will be published elsewhere. The function of thylakoid protein phosphorylation is not known but it may be involved in establishing the high energy state of the thylakoids in state 1-state 2 transitions¹⁻³. These transitions are part of the mechanism whereby the chloroplast regulates energy flux from light-harvesting chlorophyll molecules to ensure equal electron flow through the two photosystems. This regulation may involve the binding of divalent metal cations to the chlorophyll *a/b* binding protein^{18,19}. It is likely that phosphorylation of this protein would alter both its cation binding properties and its interaction with the two photosystems.

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- ¹ Barber, J. *The Intact Chloroplast* (Elsevier, Amsterdam, 1976).
- ² Govindjee *Bioenergetics of Photosynthesis* (Academic, New York, 1975).
- ³ Anderson, J. M. *Biochim. biophys. Acta* **416**, 191-235 (1975).
- ⁴ Ryrie, I. J. & Jagendorf, A. T. *J. biol. Chem.* **246**, 582-588 (1971).
- ⁵ Giaquinta, R. T. *et al. Archs Biochem. Biophys.* **162**, 200-209 (1974).
- ⁶ Murakami, S. & Packer, L. *Biochim. biophys. Acta* **180**, 420-423 (1969).
- ⁷ Thornber, J. P. A. *Rev. Pl. Physiol.* **26**, 127-158 (1975).
- ⁸ Hall, D. O. *Nature New Biol.* **235**, 125-126 (1972).
- ⁹ Bennett, J. & Milewska, Y. in *Genetics and Biogenesis of Chloroplasts and Mitochondria* (eds Bücher, T. *et al.*) 637-640 (Elsevier/North-Holland, Amsterdam, 1976).
- ¹⁰ O'Farrell, P. H. *J. biol. Chem.* **250**, 4007-4021 (1975).
- ¹¹ Boulter, D. *et al. Biol. Rev.* **47**, 113-175 (1972).
- ¹² Chu, R. C. L. & Yasumoto, K. T. *Biochim. biophys. Acta* **89**, 148-149 (1964).
- ¹³ Clegg, J. C. S. *et al. J. gen. Virol.* **32**, 413-430 (1976).
- ¹⁴ Blair, G. E. & Ellis, R. J. *Biochim. biophys. Acta* **319**, 223-234 (1973).
- ¹⁵ Bennett, J. *Phytochemistry* **15**, 263-265 (1976).
- ¹⁶ Pai, M. S. *et al. Protoplasma* **85**, 209-218 (1975).
- ¹⁷ Ralph, R. K. *et al. Biochem. J.* **130**, 901-911 (1972).
- ¹⁸ Armond, P. A. *et al. Archs Biochem. Biophys.* **175**, 54-63 (1976).
- ¹⁹ Davis, D. J. *et al. Archs Biochem. Biophys.* **175**, 64-70 (1976).

Identification of a transformation-specific antigen induced by an avian sarcoma virus

GENETIC analyses of avian sarcoma viruses (ASV) have led to the identification of a gene, designated *src*, which encodes a product required for the initiation and maintenance of neoplastic transformation in infected fibroblasts¹⁻⁵. Because the *src* gene product has not been identified biochemically, this study was initiated to detect a transformation-specific protein, using serum from rabbits bearing ASV-induced tumours. We describe here the identification of a 60,000-MW transformation-specific antigen detectable in ASV-transformed chicken cells and ASV-induced hamster tumour cells by immunoprecipitation of radiolabelled cell extracts with serum from tumour-bearing rabbits. Moreover, the expression of this antigen is temperature dependent in chicken cells transformed by an ASV temperature-sensitive mutant in the *src* gene. The use of this antiserum may lead to the unequivocal identification and characterisation of the ASV *src* gene product and this, in turn, may lead to the elucidation of the mechanism of ASV-induced oncogenesis.

Serum from rabbits whose Schmidt-Ruppin (SR) strain ASV-induced tumours followed two different patterns of growth, labelled A and B and described in the legend of Fig. 1, was tested for the presence of antibodies directed against any non-structural proteins. Cell extracts prepared from radiolabelled normal and SR-ASV-transformed chicken cells were immunoprecipitated with these sera and also with monospecific sera directed against virion structural proteins, and analysed by sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis (Fig. 1). The proteins precipitated from SR-ASV-transformed chicken cells by serum A and B (tracks 5 and 7) having molecular weights (MW) of 76,000 (Pr76), 66,000, 53,000,

27,000 and 12,000-15,000 are the precursor and intermediate and mature cleavage products of the ASV group-specific (gs) antigens^{6,7}. The 180,000 MW virus-specific protein which precipitated with anti-polymerase IgG and

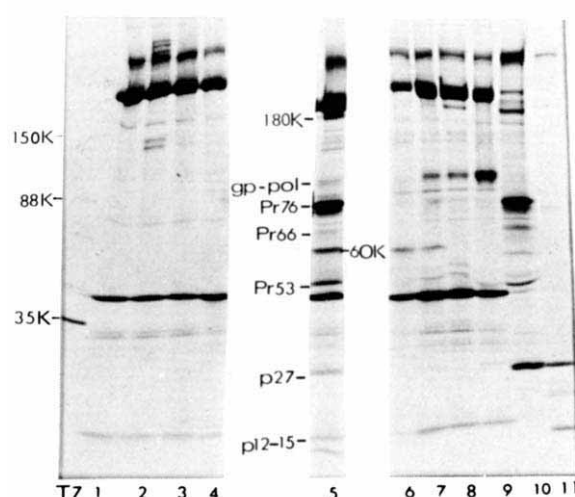


Fig. 1 Autoradiogram of SDS-polyacrylamide gel analysis of proteins immunoprecipitated from ³⁵S-methionine-labelled uninfected and SR-ASV-transformed chicken cells. Tumours were induced in rabbits by the subcutaneous and intramuscular injection of newborn New Zealand rabbits with ~10⁸ focus-forming units of purified SR-ASV (subgroup D). Palpable tumours appeared within 10-14 d in all of the nine rabbits injected. Tumour growth followed two different patterns involving either development of multiple metastases (serum A) or regression of the original tumours (serum B). In the experiments reported here, the serum was obtained 5 weeks after the animals had been inoculated with virus. Sera A and B were both found to contain antibodies to all three virion structural proteins including the viral core proteins or gs antigens, the envelope glycoprotein gp85, and the RNA-dependent DNA polymerase (J.S.B. *et al.*, unpublished observations). These antibodies were detected by immunoprecipitation of ³⁵S-labelled proteins from lysed, purified ASV and by neutralisation of virion polymerase activity and of focus formation. Adult rabbits receiving the same dose of infectious virus did not develop tumours and did not produce antibody to any virus-specific proteins. Uninfected and SR-ASV-transformed chicken embryo fibroblasts (CEF) were seeded at a density of 8 × 10⁶ cells per 100-mm Petri dish and labelled the next day for 4 h with 10 μCi ml⁻¹ ³⁵S-methionine (New England Nuclear, 400 Ci mm⁻¹) in Eagle's medium lacking methionine and supplemented with 5% calf serum (Colorado Serum Co.). The cells were washed three times with STE (150 mM NaCl, 10 mM Tris-HCl, pH 7.2, 1 mM EDTA) and lysed in RIPA buffer¹¹ (150 mM NaCl, 1% sodium deoxycholate, 1% Triton X-100, 0.1% SDS, 10 mM Tris-HCl, pH 7.2) containing 1% Trasylol, a general inhibitor of proteases¹² (FBA Pharmaceuticals, New York) and centrifuged at 90,000g for 30 min. The supernatant was divided into samples of equal volume and incubated with 10 μl of the pertinent antiserum. After 30 min at 4 °C, 200 μl of a 10% suspension of the protein A-containing bacterium, *Staphylococcus aureus*, strain Cowan I were added to adsorb the immune complexes as described by Kessler¹³. After four washes with RIPA buffer, the bacteria were resuspended in sample buffer (0.07 M Tris-HCl, pH 6.8, 11.2% glycerol, 3% SDS, 0.01% bromophenol blue, 5% β-mercaptoethanol), heated at 95 °C for 1 min, pelleted, and the supernatant was electrophoresed through a discontinuous SDS-polyacrylamide slab gel system using the buffer systems described by Laemmli¹⁴. The stacking gel contained 4.2% acrylamide, 0.1% bis-acrylamide (pH 6.8). The separation gel was a 5-15% gradient of polyacrylamide (38:1 acrylamide:bis-acrylamide), 11 cm long. Gels were stained in 0.1% Coomassie blue in 50% trichloroacetic acid for approximately 1 h and destained in several changes of 10% acetic acid, 5% methanol. The dried gels were exposed to Dupont Cronex-4 medical X-ray film. Tracks 1-3 display the polypeptides immunoprecipitated from uninfected CEF with: 1, normal rabbit serum; 2, serum B; 3, serum A. Tracks 4-10 display polypeptides immunoprecipitated from SR-ASV-transformed CEF with: 4, normal rabbit serum; 5, serum B; 6, serum B which was incubated with 125 μg of purified disrupted SR-ASV before addition of the cellular extract; 7, serum A; 8, anti-RAV-0 DNA polymerase IgG; 9, anti-gp85 serum; 10, anti-p27 serum. Track 11 contains ³⁵S-methionine-labelled ASV. T7 virion proteins are included as molecular weight markers.

anti-p27 serum as well as with sera A and B has been identified as the product of genes which encode the gs antigens and DNA polymerase⁵. The glycoprotein, gp85, migrates on this gel with the same mobility as the viral polymerase. An additional virus-specific protein with a molecular weight of 60,000 is also precipitated by both sera. This protein is not precipitated by antibody against any of the ASV structural proteins, nor is it precipitated from normal chicken cells by any sera tested. Incubation of serum B with disrupted virus before the addition of the labelled cell extract blocks the immunoprecipitation of all the virus-specific proteins except that of the polypeptide of molecular weight 60,000 (track 6). The fact that the intensity of this antigen is identical with and without the block suggests that its immunoprecipitation is not dependent on antibodies against any of the ASV structural proteins.

Figure 2 (tracks 4, 5, and 6) displays the polypeptides immunoprecipitated from a radiolabelled extract of a cloned line of SR-ASV-induced Syrian hamster tumour cells (SR-SHC/C1A). These proteins are co-electrophoresed with immunoprecipitates from SR-ASV-transformed chicken cells (tracks 1, 2, and 3). Serum B precipitated two virus-specific proteins from SR-SHC/C1A cells. One protein co-migrates with the Pr76 precipitated from SR-ASV-transformed chicken cells (track 2). No mature gs antigens are detectable in these hamster tumour cells, which suggests that Pr76 is not processed normally. The only other virus-specific protein precipitated from this hamster tumour cell line by serum B is a protein which co-migrates with the 60K protein immunoprecipitated from chicken cells. This polypeptide is not precipitated by antibody to any structural proteins, nor is it immunoprecipitated from normal Syrian hamster cells by any sera tested. When disrupted virus is incubated with serum B before the addition of the labelled cell extract (track 6), the precipitation of Pr76 is completely blocked, whereas the intensity of the 60K band is identical to

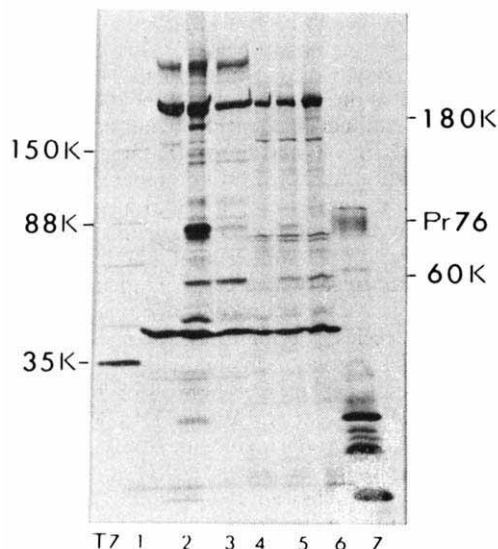


Fig. 2 Autoradiogram of SDS-polyacrylamide gel analysis of proteins immunoprecipitated from SR-ASV-transformed chicken and hamster cells. SR-SHC/C1A cells were obtained by trypsinisation of a tumour induced by injection of a newborn Syrian hamster with SR-ASV. Cells were labelled with ³⁵S-methionine, immunoprecipitated and electrophoresed as described in the legend to Fig. 1. The cells and serum used are the following: 1, ASV-transformed CEF, normal serum; 2, ASV-transformed CEF, serum B; 3, ASV-transformed CEF, serum B blocked with 125 µg disrupted virus; 4, SR-SHC/C1A, normal serum; 5, SR-SHC/C1A, serum B; 6, SR-SHC/C1A, serum B blocked with 125 µg of disrupted virus. Track 7 contains ³⁵S-methionine-labelled ASV-virion proteins.

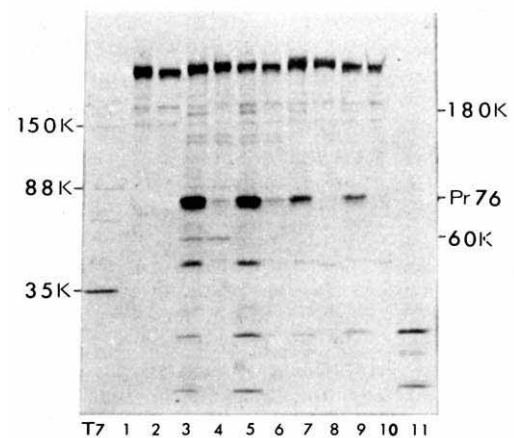


Fig. 3 Autoradiogram of SDS-polyacrylamide gel analysis of proteins immunoprecipitated from ³⁵S-methionine-labelled chicken cells transformed by the SR-ASV mutant NY68 (subgroup A). CEF cells were transformed with NY68 (ref. 4) and maintained at 35 °C. Transformed cells were trypsinised and plated at 35 °C or 41 °C at a density of 8×10^6 cells per 100-mm Petri dish and 16 h later the cultures were labelled for 1 h with 20 µCi ml⁻¹ ³⁵S-methionine, immunoprecipitated and analysed as described in Fig. 1. The temperature of incubation of NY68-transformed cells and the serum used for immunoprecipitation are the following: 1, 35 °C, normal serum; 2, 41 °C, normal serum; 3, 35 °C, serum B; 4, 35 °C, serum B blocked with disrupted virus; 5, 41 °C, serum B; 6, 41 °C, serum B blocked with disrupted virus; 7, 35 °C, anti-p27 serum; 8, 35 °C, anti-p27 serum blocked with disrupted virus; 9, 41 °C, anti-p27 serum; 10, 41 °C, anti-p27 serum blocked with disrupted virus. Track 11 contains ³⁵S-methionine-labelled ASV virion proteins.

that in track 5. The 60K protein is also detectable in another line of hamster tumour cells derived from a tumour induced by SR-ASV in the inbred LSH strain of hamsters.

The presence of an antigen having identical molecular weights in two different species of ASV-transformed cells suggests that this antigen is ASV transformation specific. This conclusion is supported by the finding that cells infected with a SR transformation-defective mutant or with RAV-50 or Carr-Zilber Associated virus (CZAV), both subgroup D viruses, do not contain the 60K protein. The expression of this transformation-specific antigen was also examined in cells infected with the SR-ASV temperature-sensitive (*ts*) mutant, NY68 (ref. 4) (subgroup A), which is defective in the initiation and maintenance of transformation at 41 °C, although virus replication is normal at this temperature. Figure 3 shows that the 60K protein was barely detectable in NY68-transformed cells grown at 41 °C relative to the 60K band from cells cultured at 35 °C. Equivalent amounts of the other virus-specific proteins such as the gs antigens and their precursors (180K, Pr76, Pr53, p27, and p12-15) were immunoprecipitated from cells grown at both temperatures, indicating that overall virus-specific protein synthesis at the two temperatures was the same and suggesting that the presence of the 60K protein is transformation dependent.

The use of serum from rabbits bearing ASV-induced tumours has allowed the detection of a 60,000 MW protein which has the following properties of an ASV transformation-specific antigen. (1) This protein is present in two different species of cells transformed by ASV. It seems unlikely that a virus-induced, cell-coded protein from two such widely divergent species would have identical molecular weights. Tryptic peptide fingerprints of the 60K proteins from transformed hamster and chicken cells are being prepared to characterise these

two proteins further. A 60K polypeptide has been synthesised in reticulocyte cell-free extracts using sub-genomic regions of the ASV genome as a mRNA (ref. 8). Experiments are in progress to ascertain whether it is the product of the ASV *src* gene. (2) The 60K polypeptide is not detectable in cells infected with non-transforming leukemia viruses (RAV-50 and CZAV) of the same subgroup as the tumour-inducing virus or with a SR transformation-defective virus. (3) The amount of 60K protein is greatly reduced at the non-permissive temperature in cells transformed by a mutant, NY68, temperature sensitive in the production of the *src* gene product. This suggests that the 60K protein could be the product of the mutant gene and that the polypeptide loses its antigenicity when denatured at 41 °C and is unable to react with the antibody or that the defective protein may be degraded more rapidly at 41 °C than the normal protein and thus less is detected in the conditions used here. Accelerated degradation of the protein products of *ts* genes at the non-permissive temperature has been demonstrated with mutants of vesicular stomatitis virus and of SV40 (refs. 9, 10). Alternatively, the 60K protein could be a virus-induced coded protein, and its reduced immunoprecipitation at 41 °C may merely reflect host changes secondarily associated with the loss of transformation. All of these possibilities are being investigated.

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- ¹ Vogt, P. K. *Virology* 46, 939-946 (1971).
- ² Biggs, P. M., Milne, B. S., Graf, T. & Bauer, H. J. *gen. Virol.* 18, 399-403 (1973).
- ³ Toyoshima, K. & Vogt, P. K. *Virology* 39, 930-931 (1969).
- ⁴ Kawai, S. & Hanafusa, H. *Virology* 46, 475-479 (1971).
- ⁵ Martin, G. S. *Nature* 227, 1021-1023 (1970).
- ⁶ von der Helm, K. *Proc. natn. Acad. U.S.A.* 74, 911-915 (1977).
- ⁷ Eisenman, R., Vogt, V. M. & Diggelmann, H. *Cold Spring Harbor Symp. quant. Biol.* 39, 1067-1076 (1974).
- ⁸ Purchio, A. F., Erikson, E. & Erikson, R. L. *Proc. natn. Acad. Sci. U.S.A.* (in the press).
- ⁹ Krieger, D., Lodish, H. F. & Baltimore, D. *J. Virol.* 21, 1140-1148 (1977).
- ¹⁰ Tegtmeyer, P., Schwartz, M., Collins, J. K. & Rundell, K. *J. Virol.* 16, 168-178 (1975).
- ¹¹ Gilead, Z. *et al. Nature* 264, 263-266 (1976).
- ¹² Biscid, G. *Acta Pharmac. Toxicol.* 28, 225-232 (1970).
- ¹³ Kessler, S. W. *J. Immun.* 115, 1617-1624 (1975).
- ¹⁴ Laemmli, U. K. *Nature* 227, 680-685 (1970).

Structures of benzo(a)pyrene-nucleic acid adducts formed in human and bovine bronchial explants

PUBLICATION by Sims *et al.* of evidence that the 7,8-dihydrodiol-9,10-oxide of benzo(a)pyrene (BP) is a metabolic intermediate in the covalent binding of this ubiquitous polycyclic aromatic hydrocarbon to DNA in hamster embryo cells¹ was followed by many related publications². Grover *et al.*³ also obtained evidence that the same derivative is involved in the binding of BP to the DNA of human bronchial explants, although details of the specific isomer involved and of the structure of the adduct were not reported. We describe here studies on RNA and DNA adducts formed by human bronchial explants and provide evidence that the structures of the major adducts are similar to those formed in the analogous bovine system.

Two stereoisomers of BP 7,8-dihydrodiol-9, 10-oxide (BPDE) have been synthesised. In isomer I, the 7-hydroxyl and 9,10-oxide groups are on the opposite sides of the plane of the ring system while in isomer II they are on the same side. Each stereoisomer has

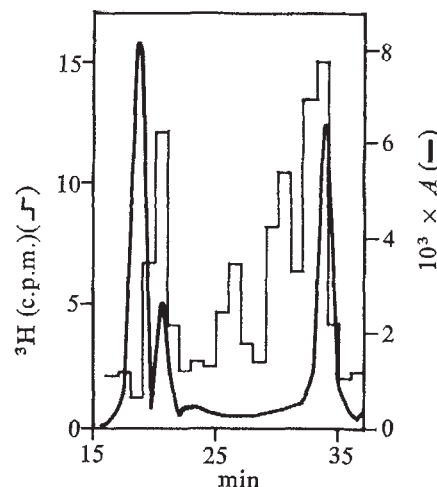


Fig. 1 HPLC profile of RNA adducts formed by human bronchial explants incubated with ³H-BP. Human bronchial explants were incubated for 24 h with ³H-BP (ref. 20). The tissue was then homogenised with a Polytron PT 10-20-350D homogeniser in 3 ml of 10 mM Tris-HCl, 0.4 M NaCl, 2 mM EDTA (pH 7.9). Sodium dodecyl sulphate (SDS) (30 µl 20%) and proteinase K (0.5 ml, 2 mg ml⁻¹) were then added and the mixture incubated overnight to dissolve the tissue. The solution was then extracted several times with water saturated phenol containing 0.1% SDS and 0.1% 8-hydroxyquinoline and once with chloroform-amy alcohol (3:1) before the addition of 0.03 volumes of 2 M sodium acetate (pH 5.0) and precipitation with 2 volumes of ethanol. The ethanol precipitation was repeated and followed by the addition of 50 A₂₅₀ units of poly (G) previously modified by reaction with BPDE I (ref. 12) and then hydrolysis of the RNA in 0.3 M NaOH overnight at 37 °C. The samples were neutralised with acetic acid and chromatographed on Sephadex LH 20 columns. The DNA eluted in the void volume and the modified ribonucleotides eluted with 40% methanol. The latter were treated with alkaline phosphatase overnight before separation by Sephadex LH 20 chromatography, this time eluting with 80% methanol. The modified nucleosides were then resolved by HPLC on Zorbax ODS columns operated at 2,500 pounds per square inch at 50 °C, with 30% methanol in water for elution. Adducts resulting from the *in vitro* reaction of BPDE with poly (G) were detected by ultraviolet absorbance at 280 nm and *in vitro* samples by their radioactivity.

two enantiomers which have also been prepared^{4, 6}. We have previously published the complete structure and stereochemistry of a major RNA adduct formed in bovine bronchial explants exposed to BP (refs 5, 7, 8) and shown that it is derived from the 7R,8S,9R,10R enantiomer of BPDE (designated 7R-BPDE I) by the *trans* addition of the N² amino group of guanine to the 10 position of the dihydrodiol oxide. Our assignment of the absolute stereochemistry of BP 7,8-dihydrodiol obtained during these studies has been confirmed by Yagi *et al.*⁴. Evidence that RNA (ref. 9) and DNA adducts^{10, 11} are formed from both isomers I and II in other tissues and species, and that these involve the formation of similar guanine adducts, has been obtained.

After exposure of explants of human or bovine bronchial segments to ³H-BP, cellular RNA and DNA were isolated and digested to release the modified ribo- and deoxyribonucleosides. The latter were separated by Sephadex LH-20 chromatography and then analysed by high pressure liquid chromatography (HPLC) using appropriate markers synthesised *in vitro*.

Figure 1 shows the HPLC profile obtained when human RNA was digested and cochromatographed with marker nucleoside adducts formed *in vitro* by the reaction of BPDE I with poly G. The profile of the human RNA *in vivo* products is similar to that reported for bovine RNA samples⁵, although the minor products are more pronounced with the human material. The digest of human RNA revealed four distinct radioactive peaks, designated H-RNA 1-4 (Fig. 1 and Table 1). We have previously reported that BPDE I and BPDE II can form adducts *in vitro* with guanosine, cytidine and adenosine¹². Table 1 indicates that during HPLC the radioactive peaks eluted in the same region as some of these guanosine and cytidine adducts. The adenosine adducts formed *in*

vitro elute much later and very little radioactivity was detected in this region. By comparing the elution positions, before and after acetylation, of the radioactive peaks to the elution positions of the guanosine and cytidine adducts formed *in vitro*, we could conclude that H-RNA peaks 1 and 4 coincided with 7R-BPDE I-guanosine adducts GI-2 and GI-3, respectively; H-RNA peak 3 with a BPDE II-guanosine adduct GII-2; and H-RNA 2 with a BPDE I-cytidine adduct CI-3 (Table 1). A major peak, H-RNA 4, coincides with the major bovine RNA adduct whose structure and stereochemistry has been previously elucidated^{5,7,8}. This compound results from *trans* addition of the 2 amino group of guanine to the 10 position of the 7R enantiomer of BPDE I. H-RNA I also is derived from 7R BPDE I (ref. 8) and represents the analogous compound formed by *cis* addition (unpublished results and ref. 9). The predominance of adducts derived from a specific enantiomer of BPDE I suggests that both bovine^{7,8} and human tissue oxidise BP with a considerable degree of stereospecificity. Separate studies¹³⁻¹⁵ also indicate considerable stereospecificity in the metabolism of BP. Based on elution order and identity of circular dichroism (CD) spectra ($\Delta_{250} = -93$), peak GII-2 (Table 1) corresponds to an adduct whose structure has been previously elucidated by Koreeda *et al.*^{9,16}. It results from *trans* addition of the 2 amino group of guanine to the 10 position of 7S BPDE II. Since HRNA-3 is

Table 1 Separation of BP-nucleoside adducts and their corresponding acetates by HPLC

Compound	Retention time		Compound	Retention time	
	Nucleoside	Acetate		Nucleoside	Acetate
HRNA-1	20	21	CII-1	21	18
HRNA-2	26	23	CII-2	29	20
HRNA-3	31	34	CII-3	42	—
HRNA-4	33	37	CII-4	44	—
GI-1	18	27	CII-5	55	—
GI-2	20	21	CI-1	17	19
GI-3	33	37	CI-2	20	13
GII-1	23	23	CI-3	26	23
GII-2	31	34	CI-4	27	14
GII-3	33	28	CI-5	31	15
GII-4	58	—	CI-6	44	12

The nucleoside adducts were prepared and separated on HPLC as described in Fig. 1. Data are retention times of the human bronchial RNA (HRNA) adducts formed *in vivo*, and of guanosine (G) and cytidine (C) adducts formed *in vitro* by reaction with BPDE isomer I or II. Numbers 1, 2, 3, 4 refer to the order of elution of individual peaks from column. After elution individual peaks were acetylated (pyridine, acetic anhydride, 18 h room temperature) and re-run on HPLC using 55% methanol in water as solvent. The underlines indicate *in vitro* products which were cochromatographic with specific human RNA adducts. Further evidence for the chromatographic identity of HRNA-2 and 3 with the *in vitro* markers (CI-3 and GII-2) was obtained when their acetates were run on HPLC with 50% methanol as eluent. In these conditions slight isotopic separations were observed, similar to those previously reported²¹.

cochromatographic with GII-2 this indicates that there is some synthesis of BPDE II by human tissue.

The situation with DNA from both human and bovine samples was much simpler than with RNA. Following enzymatic digestion of the DNA samples, 80% of the modified deoxynucleosides eluted from the HPLC columns essentially as a single component, both before and after acetylation (Fig. 2). (The remaining 20% may be incompletely digested materials, since it eluted in the void volume of the Sephadex LH-20 column, and not been further characterised). With both the human and bovine material, the single radioactive peak cochromatographed with one of the deoxyguanosine-BPDE I derivatives, and not with deoxyguanosine derivatives obtained with isomer II (Fig. 2a). Further evidence is provided in Fig. 2b which indicates that after acetylation the radioactive product was clearly separated from the acetylated isomer II adducts.

The deoxyguanosine adduct formed *in vitro* which corresponded to the major DNA adduct formed in human and bovine tissues was then isolated in a quantity sufficient for CD spectra (Fig. 3). The spectrum of this adduct was identical to that previously reported for the major guanosine adduct^{7,8}. This is consistent with the fact that the presence or absence of the 2'-hydroxyl group of the sugar residue should have little or no influence on the CD spectrum. The CD spectrum is extremely complex since it consists of extrema due to the perturbed pyrene and guanosine chromophores as well as the spatial interactions between the two chromophores, the latter being predominant. The identity of the CD spectra of the deoxyguanosine and guanosine adducts and the fact that the absolute stereochemistry of the BPDE I must be 7R (refs 15, 17) indicates that, except for the 2'-hydroxyl group, the structures and absolute stereochemistry of the two adducts are identical (see Fig. 3). As in the case of the RNA adduct^{6,7-9}, the 2-amino group of guanine attacks position 10 of the 7R enantiomer of BPDE I through *trans* opening of the epoxide.

Both human and bovine RNA samples displayed minor adducts, the analogues of which were not detected in the DNA samples. The probable origin of two of these is described above, others were present in insufficient amount for identification. Possible explanations for the greater heterogeneity of adducts of RNA include the following. (1) The double stranded property of native DNA may contribute selectivity to its modification. (2) Both BPDE isomers I and II may be formed in the cytoplasm, but due to the greater instability of isomer II (ref. 18) it may react only with cytoplasmic RNA and not reach the nuclear DNA. (3) There

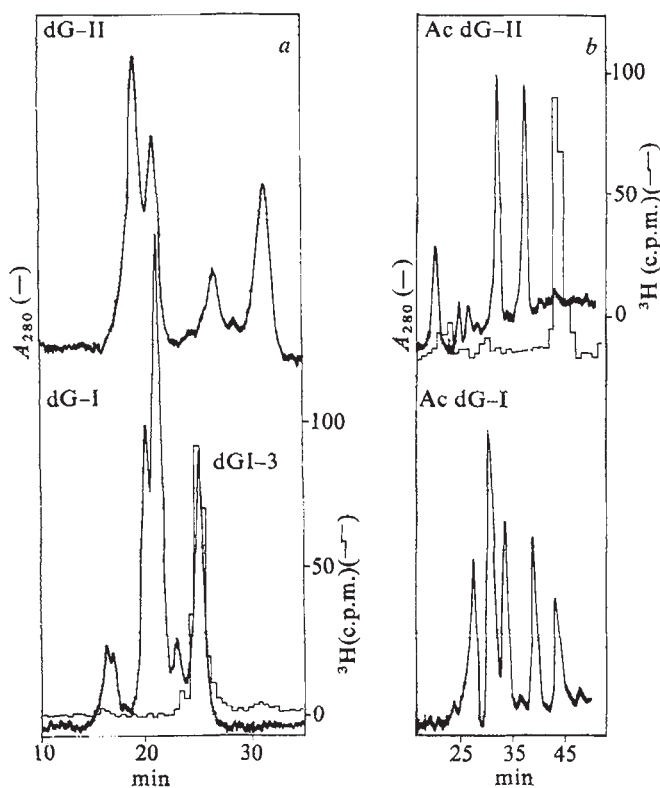


Fig. 2 HPLC profiles of human and bovine DNA adducts formed by bronchial explants incubated with ³H-BP. The radioactive human or bovine DNA which eluted in the void volume of the Sephadex LH 20 column (see Fig. 1 legend) was digested with DNase I, snake and spleen phosphodiesterases and alkaline phosphatase¹². The modified deoxynucleosides were isolated by Sephadex LH 20 chromatography and co-chromatographed on HPLC with ultraviolet markers synthesised *in vitro*. a, Compares the HPLC profile of the human ³H-deoxynucleoside adducts to that of deoxyguanosine adducts synthesised *in vitro* by reaction of 5' dGMP with either BPDE I (dG-I) or BPDE II (dG-II). HPLC conditions: Lichrosorb C18, 5 μ m, 3.2 $\times$ 250 mm column (Rainin), 2,500 pounds per square inch, 45% methanol, 50 $^{\circ}$ C. The major (>90%) *in vivo* DNA adduct eluted with a dG adduct (dGI-3) formed from BPDE I. b, Compares the HPLC profile of the acetates of ³H-bovine deoxynucleoside adducts to those of the acetates of dG-II and dG-I. HPLC conditions: Zorbax ODS, 2.1 $\times$ 250 mm column (Dupont); 2,500 pounds per square inch, linear gradient of 50–65% methanol in 50 min, 50 $^{\circ}$ C. A single radioactive *in vitro* product was obtained (upper panel, b) which, when reanalysed on HPLC with 55% methanol as solvent, was cochromatographic with the acetate of dGI-3. Similar results were obtained with the acetate of the major ³H-human deoxynucleoside adduct.

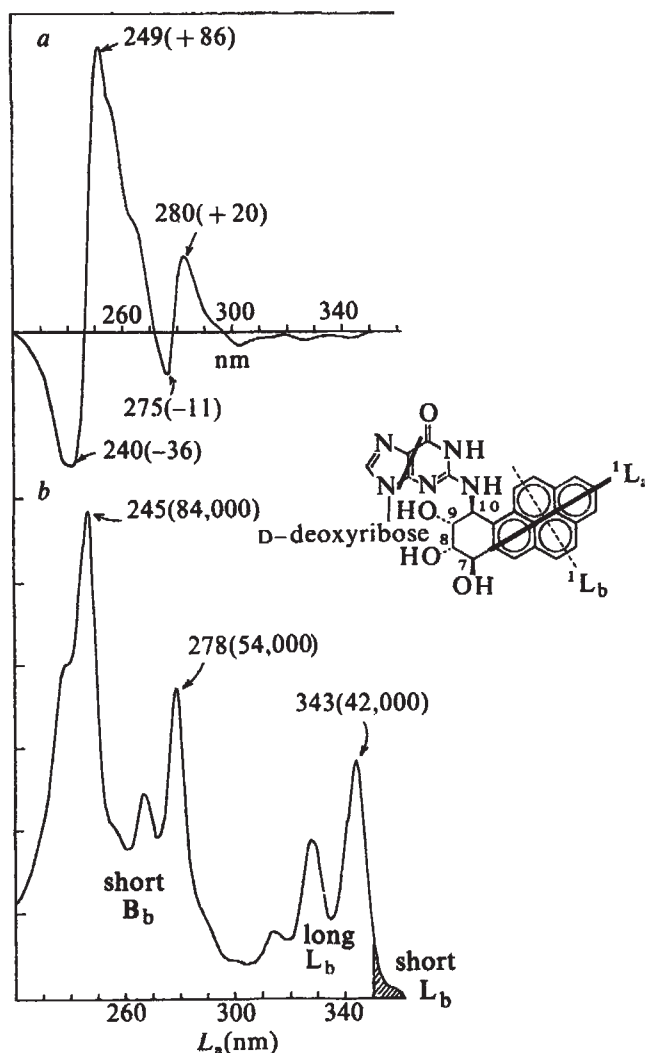


Fig. 3 *a*, CD and *b*, ultraviolet spectra of dG1-3 (the product corresponding to the major *in vitro* DNA adduct from human and bovine bronchial explants). The sample was prepared and purified by HPLC as described in Fig. 2. Spectra in 50% water-methanol. The CD $\Delta\epsilon$ values are 30% lower than previously reported for guanine adducts⁷ because of a computational error in the previous data. The ultraviolet spectrum of the adduct is dominated by the strong bands of the pyrene chromophore, the transitions of which are: shoulder (shaded area) at 350–360 nm, $1L_b$, short axis; 310–355 nm, $1L_a$, long axis; 260–290 nm, $1B_b$, short axis²². These interact with the guanine transitions at 260–270 nm, as shown by the solid line in the guanine structure, to give rise to the complex CD spectrum.

may exist a separate nuclear monooxygenase system¹⁹ which has greater stereospecificity than the cytoplasmic system in the synthesis of BPDE. Further studies are required to distinguish these possibilities.

Our investigations on the major BP metabolite responsible for covalent binding to cellular DNA in human tissue, and our evidence for the structure of the DNA adduct which is formed may contribute to the understanding of the possible human hazards associated with environmental exposure to BP and other polycyclic aromatic hydrocarbons.

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- ¹ Sims, P., Grover, P. L., Swaisland, A., Pal, K. & Hewer, A. *Nature* **252**, 326–328 (1974).
- ² *Inst. Sci. Infor., Citation Index*, 1975 and 1976.
- ³ Grover, P. L., Hewer, A., Pal, K. & Sims, P. *Int. J. Cancer* **18**, 1–6 (1976).
- ⁴ Yagi, H. *et al. J. Am. chem. Soc.* **99**, 2358–2359 (1977).
- ⁵ Weinstein, I. B. *et al. Science* **193**, 592–595 (1976).
- ⁶ Harvey, R. G. & Cho, H. *Analyt. Biochem.* **80**, (in the press).
- ⁷ Jeffrey, A. M. *et al. J. Am. chem. Soc.* **98**, 5714–5715 (1976).
- ⁸ Nakanishi, K. *et al. J. Am. chem. Soc.* **99**, 258–260 (1977).
- ⁹ Moore, P. D. *et al. Am. chem. Soc. Symp. Ser.* **44**, 127–154 (1977).
- ¹⁰ King, H., Osborne, M., Beland, F., Harvey, R. & Brookes, P. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2679–2681 (1976).
- ¹¹ Remsen, J., Jerina, D., Yagi, H. & Cerrutti, P. *Biochem. biophys. Res. Commun.* **74**, 934–940 (1977).
- ¹² Jennette, K. W. *et al. Biochemistry* **16**, 932–938 (1977).
- ¹³ Yang, S. K., McCourt, D. W., Leutz, J. C. & Gelboin, H. V. *Science* (in the press).
- ¹⁴ Yang, S. K., McCourt, D. W., Roller, P. P. & Gelboin, H. V. *Proc. natn. Acad. Sci. U.S.A.* **73**, 2594–2598 (1976).
- ¹⁵ Thakker, D. R. *et al. Chem.-Biol. Interact.* **16**, 281 (1977).
- ¹⁶ Koreeda, M., Moore, P. D., Yagi, H., Yeh, H. J. C. & Jerina, D. M. *J. Am. chem. Soc.* **98**, 6720–6723 (1976).
- ¹⁷ Yang, S. K., Gelboin, H. V., Trump, B. F., Autrup, H. & Harris, C. C. *Cancer Res.* **37**, 1210–1215 (1977).
- ¹⁸ Yagi, H., Hernandez, O. & Jerina, D. M. *J. Am. chem. Soc.* **97**, 6881–6883 (1975).
- ¹⁹ Khandwala, A. S. & Kasper, C. B. *Biochem. biophys. Res. Commun.* **57**, 1241–1246 (1973).
- ²⁰ Harris, C. C. *et al. Cancer Res.* **36**, 1011–1018 (1976).
- ²¹ Jeffrey, A. M. & Fu, P. P. *Analyt. Biochem.* **77**, 298–302 (1977).
- ²² Becker, R. S., Singh, I. S. & Jackson, E. A. *J. chem. Phys.* **38**, 2144–2171 (1963).

Nucleotide sequence of *Bombyx mori* L. tRNA₁^{Gly}

THE functional adaptation of the tRNA population in the posterior silk gland of the silkworm *Bombyx mori* L. during growth and secretion of fibroin¹ is the result of changes in the predominant isoaccepting tRNA species to accord with the frequency of various codons in fibroin mRNA (refs 2–4). tRNA adaptation to fibroin biosynthesis can be demonstrated by polyacrylamide gel electrophoresis maps. The main glycine codons GGU and GGA of fibroin mRNA (ref. 3) are translated by means of two prevalent iso-tRNA^{Gly} species: iso-tRNA₁^{Gly} decodes GGU and GGC whereas iso-tRNA₂^{Gly} decodes GGA (refs 4–6). The glycine tRNA₁, the first sequenced tRNA from an insect⁶, has 74 nucleotides and relatively few modified residues⁷: one thymidine, one pseudouridine, one dihydrouridine, one 1-methyl adenine, 1-methyl guanosine, four 5-methyl cytidine and one unusual 2'-O-methyl uridine in the acceptor stem. It carries the expected GCC anticodon⁴. Comparison with three other homodecoding tRNA_{GCC}^{Gly} species reveals a high degree of homology.

The functional adaptation of tRNA to the amino acid composition of proteins being synthesised is best demonstrated by studying the quantitative changes of well-defined iso-tRNA species during development of the silk gland of the lepidopteran *Bombyx mori* L., which produces massive amounts of fibroin and sericin in the posterior and middle part respectively^{1,2}. The observed changes in tRNA species during the last larval stage before cocoon spinning are closely associated with the codon frequencies of glycine, alanine and serine in the rapidly accumulating fibroin mRNA, mainly GGU: GGA in a ratio of 1.4:1, GCU and UCA (refs 2–5). The sequence of the prominent tRNA₁^{Gly} species supports our predictions, based on triplet binding, of the presence of a GCC anticodon able to decode GGU and GGC codons^{4,6}. This remarkable iso-tRNA₁^{Gly} represents 20 ± 3% of total tRNA at the end of the secretion phase of fibroin in the posterior silk gland whereas iso-tRNA₂^{Gly} able to decode GGA and GGG accounts for 15 ± 2%. Their ratio matches that of the corresponding codons in fibroin mRNA (refs 2, 8).

The posterior silk glands from hybrids of European strains 200 and 300 were dissected at the end of the last larval instar. Transfer

RNA was extracted as previously described⁹ and tRNA₁^{Gly} purified by a 1,500 transfer counter-current distribution to 80% and subjected to a final DEAE-cellulose chromatography at 65 °C 7 M urea^{6,7}. This tRNA species is strongly polar, because of a low ratio of adenine to pyrimidine⁷, and has been identified by two-dimensional polyacrylamide gel electrophoresis as shown in Fig. 1. Co-electrophoresis of tRNA₁^{Gly} from the middle silk gland secreting sericin and from the carcass rich in a chitin-associated protein shows that they have identical mobility. The pancreatic and T₁ RNase digestions, separation of the oligonucleotides on neutral and acidic DEAE-cellulose columns and high voltage electrophoresis, nucleotide analysis by thin-layer chromatography and all subsequent steps for further sequence analysis were carried out as described previously¹⁰. The identity of oligonucleotides has been checked again with ³²P-tRNA₁^{Gly} isolated from two-dimensional gel and subjected to a mini-fingerprint⁸: electrophoresis on cellulose acetate strip at pH 3.5 followed by homochromatography on a PEI-cellulose thin-layer plate for the second dimension. A partial digestion with T₁ RNase, which yields mainly two halves, allows us to reconstruct unambiguously a unique sequence.

The complete sequence of *B. mori* tRNA₁^{Gly} or tRNA_{GCC}^{Gly} is shown in Fig. 2 arranged in the cloverleaf model. It belongs to class I of tRNA characterised by four base pairs in the dihydrouridine stem and a short variable loop with four nucleotides. In addition to

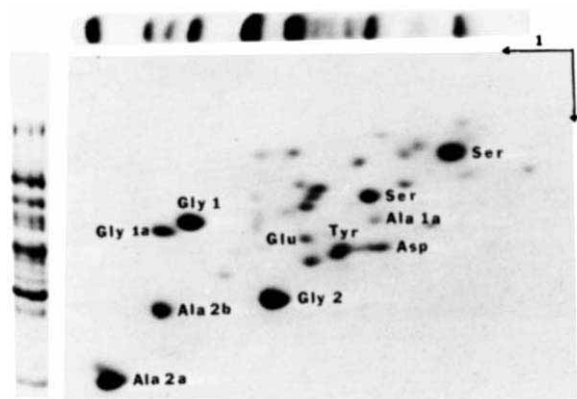


Fig. 1 Two-dimensional polyacrylamide gel electrophoresis of posterior silk gland tRNA. One A₂₆₀ unit of total tRNA from the posterior silk gland of *B. mori* 7-day-old larvae is applied to a 9.6% acrylamide gel, 7 M urea, Tris-borate buffer pH 8.2 and run over 35 cm for 45 h at 450 V (first dimension from right to left). The gel strip is cut out and embedded atop 20% acrylamide, 4 M urea prepolymerised gel slab. The electrophoresis is run in the same buffer for 60 h at 120 V (second dimension from top to bottom). tRNA spots are located after staining with 0.2% methylene blue solution in 2% sodium acetate buffer pH 5.0 or by autoradiography using ³²P-labelled tRNA. Identification is performed either by acylation and deamination with nitrous acid to stabilise the ester bond or by using purified samples of tRNA species from counter-current distribution and/or BD-cellulose chromatography^{8,16}.

the standard 23 invariant and semi-variant residues responsible for the maintenance of the tertiary structure of the macromolecule¹¹ shown in the composite diagram of Fig. 3, the tRNA₁^{Gly} reveals some characteristic features already mentioned⁶. The occurrence of a Um in position 4 of the acceptor stem seems to be a property of eukaryotic tRNA_{GCC}^{Gly}, which have been sequenced, since only tRNA₁^{Gly} species from yeast¹² and from wheat germ¹³ carry a 2'-O-methyl cytidine in the homologous position. The minor spot of tRNA₁^{Gly}, (*a* in Fig. 1), could be the non-methylated form⁸. The guanosine residue in the wobble position of the predicted GCC anticodon enables tRNA₁^{Gly} species to decode the main GGU codon found in fibroin mRNA (ref. 3). Its exposed position makes an easy partial digestion with T₁ RNase yielding two halves (a pGp 5'-terminal fragment of 33 nucleotides and the 3' end of 41 nucleotides with different compositions) useful in reconstructing

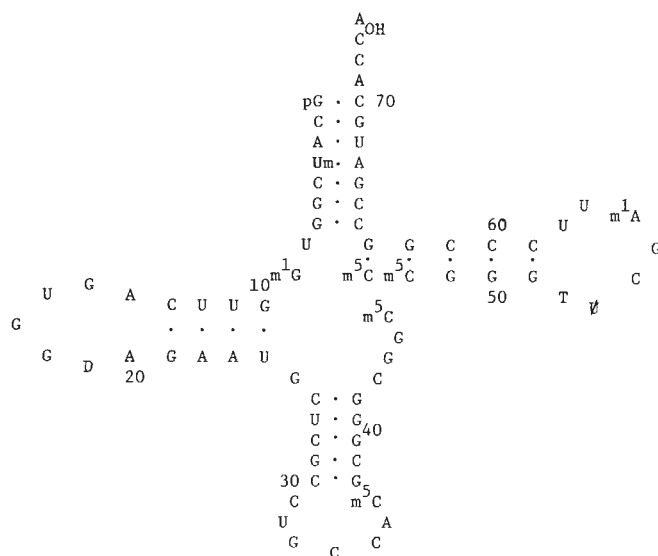


Fig. 2 Nucleotide sequence of *Bombyx mori* L. major tRNA₁^{Gly} or tRNA_{GCC}^{Gly} arranged in the cloverleaf model. Standard abbreviations are used for the nucleotides. Base pairs are represented by a dot. Specific cleavages with pancreatic and T₁ RNases yield 20 and 18 digestion products respectively. Partial T₁ digestion at the exposed guanosine in the wobble position 33 gives two halves used to reconstruct unambiguously the unique sequence. The iso-tRNA₁^{Gly} from posterior silk gland contains the GCC anticodon able to decode the main codon GGU found in fibroin mRNA³.

the total sequence. Like the other tRNA species decoding GNN triplets, the anticodon loop does not contain a hypermodified purine in position 36. The m⁵C residue at position 37 has been found in the homodecoding tRNA_{GCC}^{Gly} from wheat germ¹³ as well as in the major cytoplasmic valine tRNA of mouse myeloma cells¹⁴, which carries the homologous tetranucleotide CAM⁵CGp. The m⁵C triplet located at the junction of the short variable loop and the TΨC stem also occurs only in tRNA_{GCC}^{Gly} species of

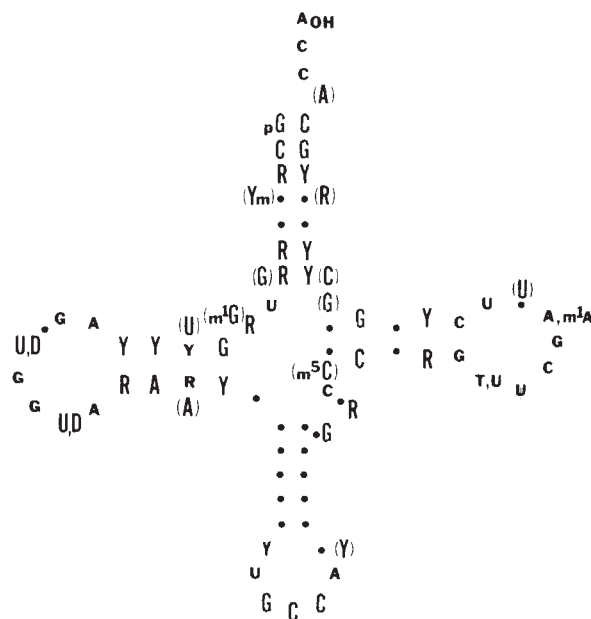


Fig. 3 Composite diagram of homodecoding tRNA_{GCC}^{Gly} species. We have included four tRNA_{Gly} species able to decode GGU and GGC: tRNA₃^{Gly} from *E. coli*¹⁷, tRNA₁^{Gly} from yeast¹², wheat germ¹³ and *B. mori*. Nucleotides not common are indicated by dot. The 23 invariant and semi-variant nucleotides are represented in small letters. R stands for purine and Y for pyrimidine. Those homologous to the four tRNA_{GCC}^{Gly} species are noted in large letters. In parentheses we have shown additional similarities between the three eukaryotic species.

higher eukaryotic organisms from wheat germ to human placenta¹⁵. The presence of four m⁵C residues specific for the preponderant iso-tRNA^{Gly}₁, in contrast to the two found in the iso-tRNA^{Gly}₂, can be used as a marker for tRNA biosynthesis studies^{7,8}. We have to point out the presence of the 'invariant' thymidine in the T Ψ C sequence is not ubiquitous with respect to the *B.mori* tRNA species. Our nucleoside and fingerprint analysis demonstrate clearly that both preponderant tRNA^{Ala}_{2a} species (specific for the posterior silk gland) and tRNA^{Ala}_{2b} (common in all silkworm tissue)¹⁶ have no thymidine^{7,8}.

Figure 3 represents the composite diagram of known homodecoding tRNA^{Gly}_{GCC} species: tRNA^{Gly}₃ from *E.coli*¹⁷, tRNA^{Gly}₁ from yeast¹², wheat germ¹³ and *B.mori*. Considering both purine residues as R and pyrimidine residues as Y, the homodecoding tRNA^{Gly}_{GCC} species so far sequenced have 71.0% homology whereas eukaryotic species alone have 77.0%. There is an increase in sequence homology from *E.coli* tRNA^{Gly}_{GCC} to yeast, wheat germ and *B.mori* corresponding to their phylogenetic relationship. Because of its great variability, however, the anticodon stem could be considered as a target susceptible to evolutionary pressure. This reinforces the presumption that the central part of the tRNA molecule is involved in the recognition sites of cognate ligase and ribosome.

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- ¹ Garel, J. P. *J. theor. Biol.* **43**, 211-225 (1974).
- ² Garel, J. P. *Nature* **260**, 805-806 (1976).
- ³ Suzuki, Y. & Brown, D. D. *J. molec. Biol.* **63**, 409-429 (1972).
- ⁴ Garel, J. P., Hentzen, D. & Daillie, J. *FEBS Lett.* **39**, 359-363 (1974).
- ⁵ Chen, G. S. & Siddiqui, M. A. Q. *Arch. Biochem. Biophys.* **161**, 109-117 (1974).
- ⁶ Garel, J. P., Dirheimer, G. & Keith, G. *Z. physiol. Chem.* **357**, 293 (1976).
- ⁷ Garel, J. P., Hentzen, D., Schlegel, M. & Dirheimer, G. *Biochimie* **58**, 1089-1100 (1976).
- ⁸ Garel, J. P., Garber, R. & Siddiqui, M. A. Q. *Biochemistry* (in the press).
- ⁹ Chavancy, G., Daillie, J. & Garel, J. P. *Biochimie* **53**, 1187-1194 (1971).
- ¹⁰ Keith, G., Roy, A., Ebel, J. P. & Dirheimer, G. *Biochimie* **54**, 1405-1415 (1972).
- ¹¹ Rich, A. & RajBhandary, U. L. *J. Rev. Biochem.* **45**, 805-860 (1976).
- ¹² Yoshida, M. *Biochem. Biophys. Res. Commun.* **50**, 779-784 (1973).
- ¹³ Marcu, K. B., Mignery, R. E. & Dedrick, B. S. *Biochemistry* **16**, 797-806 (1977).
- ¹⁴ Piper, P. W. *Eur. J. Biochem.* **51**, 295-304 (1975).
- ¹⁵ Roe, B. A., Chen, E. Y. & Tsen, H. Y. *Biochem. Biophys. Res. Commun.* **68**, 1339-1347 (1976).
- ¹⁶ Meza, L., Araya, A., Leon, G., Krauskopf, M., Siddiqui, M. A. Q. & Garel, J. P. *FEBS Lett.* **77**, 255-260 (1977).
- ¹⁷ Squires, C. & Carbon, J. *Nature new biol.* **233**, 274-277 (1971).

Melting fine structure of DNA fragments of known base sequence from ϕ X174

THERE is much experimental evidence of discrete unfolding of double-stranded DNA, revealed as fine structures in the melting profile (refs 1-4 and refs therein). Although this is believed to reflect aspects of the nature of the base sequence through the instability of AT and GC base pairs⁵, detailed quantitative analysis is hindered by factors such as the highly cooperative nature of double-helix stabilisation by base stacking and the long-range correlation of helical states through the entropy effect of the unfolded loop. Furthermore, the lack of appropriate nomenclature for the base sequence has complicated discussion. Fortunately, essentially the whole base sequence of the DNA of bacteriophage ϕ X174 has been determined⁶, and we present

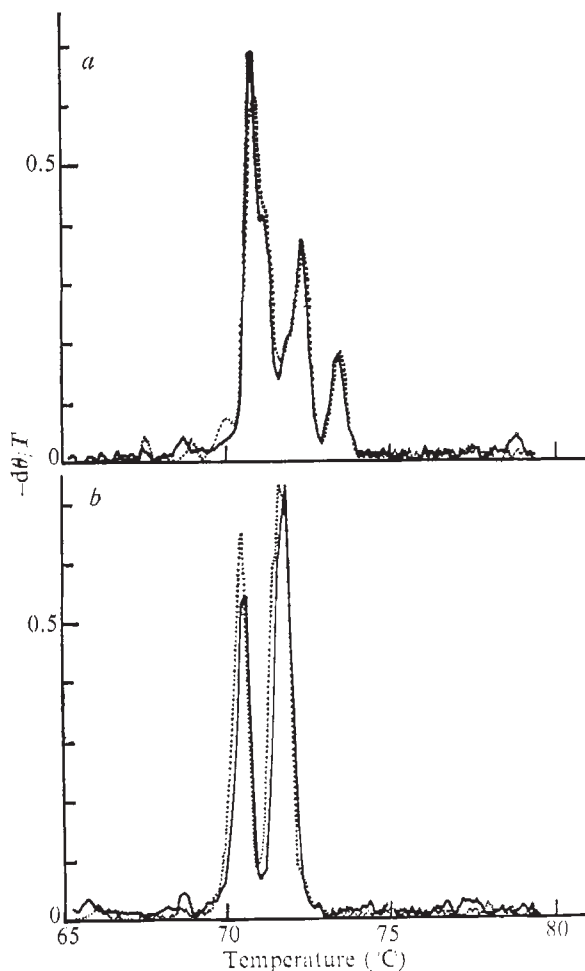


Fig. 1 Melting profiles of Y_1 -fragment (a) and Y_2 -fragment (b) of DNA of phage ϕ X174. Double-stranded, closed-circular replicative form (RFI) of ϕ X174 phage was prepared from ϕ X174 *am3*-infected *Escherichia coli* HF4704 cells as before⁷. The RFI molecules were cleaved by restriction enzyme *HapII*, and the products were separated by polyacrylamide gel electrophoresis⁸. The gels were scanned at 260 nm using a Gilford spectrophotometer 250 to define the regions of the DNA fragments. The gel regions containing the largest (Y_1) and the second largest (Y_2) fragments were excised and these fragments were extracted as in ref. 9. Melting profiles were obtained with a minicomputer-controlled on-line melting plotter¹. The displays are made in differential form as $d(1-\theta)/dT$ against T , where θ is the helix content obtained by the optical density measurement at 260 nm, and T is the temperature. The ordinate is not normalised to the fragment length. Two results of independent measurements are shown by the solid and dotted lines in each figure.

here melting profiles of two fragments obtained from the replicative form by means of the restriction enzyme *HapII*, and examine them with reference to their base sequence.

The differentiated melting profiles of fragments Y_1 (2,745 base pairs: position 3360-5375-729) and Y_2 (1,690 base pairs: position 1104-2793) measured in a $0.1 \times$ SSC aqueous solution are shown in Fig. 1a and b, which have three and two well separated peaks, respectively, with some reproducible hyperfine structure in several of the peaks. Numerical data for the characteristics of the peaks are listed in Table 1.

The base sequences of these fragments are also shown in Fig. 2a and b in the form of the distribution function $P_n(x)$ of the G+C content, x , of sections each containing n base pairs along the DNA fragment, the first section starting with the first base pair, the second with the second base pair and so on until the whole fragment is covered. If the base sequence of the fragment is purely random and sufficiently long compared with the length of the section, n , the distribution function should agree with the binomial distribution

$$P_n(x) = \frac{n!}{\{n(1-x)\}!\{nx\}!} X_0^{nx}(1-X_0)^{n(1-x)}$$

where X_0 is the G+C content of the whole fragment. The binomial distribution for a small value of n is also plotted in the same figure for comparison.

We have speculated that a peak in the melting profile corresponds to a homostability region or regions with a fairly homogeneous base sequence, because computer simulations based on the established molecular thermodynamic parameters for several randomly generated base sequences did not reproduce sharp peaks as obtained in melting experiments^{1,4}. On the other hand, Lyubchenko *et al.* have shown that a sharp fine structure can be simulated by some computer-generated random base sequences³. But, arguments based on a limited number of such sequences may not be meaningful, because a DNA molecule of 1,000 base pairs, for example, has $2^{1,000}$ varieties of base sequence.

We therefore draw the following conclusions. (1) Fragments

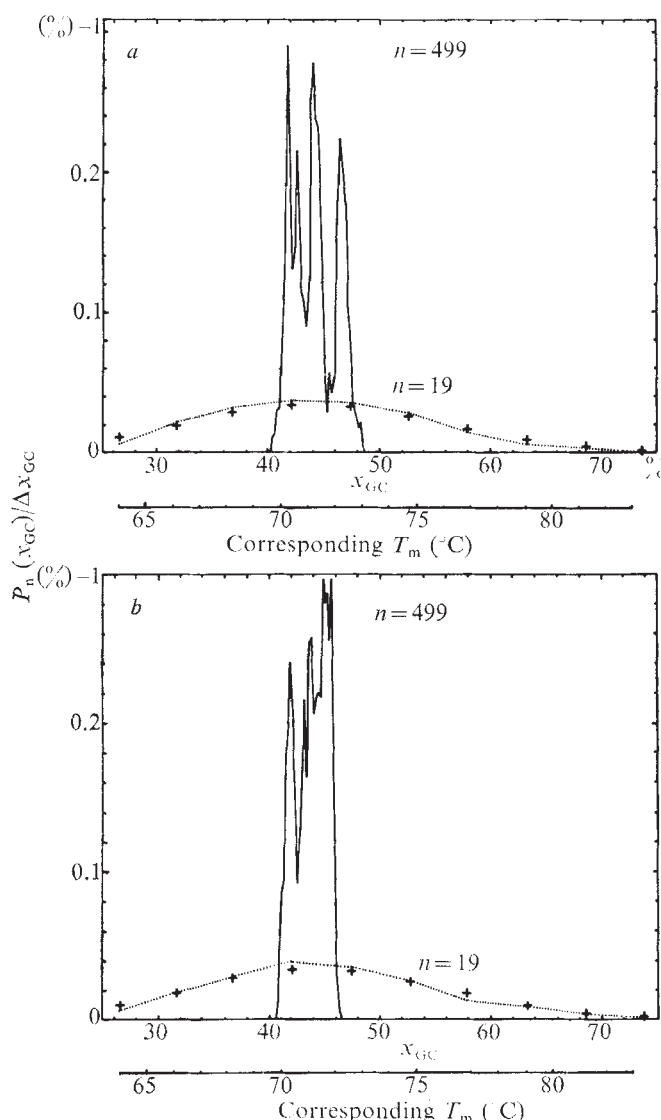


Fig. 2 Local segmental distribution of G+C content in Y₁-fragment (a) and Y₂-fragment (b) of DNA of phage ΦX174. The distribution function is expressed in terms of the G+C content, x , of sections each containing n base pairs along the DNA fragment, the first section starting with the first base pair, the second with the second base pair and so on until the whole fragment is covered. The last section, therefore starts with $(N-n+1)$ th pair where N is the total number of base pairs in the fragment. Two distributions with $n=19$ and $n=499$ are shown. The binomial distribution for $n=19$ is shown by crosses.

Table 1 Numerical data for the melting peaks

Fragment	Peak no.	$T_m(^{\circ}\text{C})$	$2\sigma^{\dagger}$	Length (base pairs)
Y ₁	1 $\ddagger$	70.8 $\pm$ 0.1	0.54	1,570
	2 $\ddagger$	72.3 $\pm$ 0.1	0.53	850
	3	73.4 $\pm$ 0.1	0.42	330
Y ₂ *	1	70.6 $\pm$ 0.1	0.39	680
	2 $\ddagger$	71.8 $\pm$ 0.1	0.56	1,010

Peaks were measured in 0.1 ssc.

* Mean value of two measurements.

$\dagger$ Full width at $0.61 \times$ maximum height.

$\ddagger$ Peak having hyperfine structure.

Y₁ and Y₂ of ΦX174 DNA have well resolved fine structures corresponding to the cooperative melting of sections containing 300–1,500 base pairs (Fig. 1). (2) The base sequences of these fragments are fairly random when observed in short lengths, 'random' being defined in terms of several criteria, such as the nearest neighbour base frequency, autocorrelation of the base sequence and the binomial distribution of the G+C content (Fig. 2). (3) The distribution of the local segmental G+C content shows some deviation from a smooth distribution when it is averaged over several hundred bases (Fig. 2). Whether this distribution is statistical or has some functional significance cannot be determined because of the small number of samples available. (4) The melting profiles seem to reflect the long-range base distributions when Figs 1 and 2 are compared. (5) Detailed analysis shows that cooperativity in the melting of DNA is much stronger than previously expected, and the value of the cooperativity parameter used by Lyubchenko *et al.*³ seems to be more appropriate than that used earlier.

It is important that ambiguous and unrealistic terminology, such as 'homogeneous' or 'random', should not be used to describe the nature of base sequences with limited length. Instead, because the melting profile of a known sequence unambiguously shows several peaks, we are working on the determination of the molecular thermodynamic parameters for the melting of DNA. A quantitative discussion on the relation between the base sequence and the melting profile will be published elsewhere.

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¹ Gotoh, O., Husimi, Y., Yabuki, S. & Wada, A. *Biopolymers* **15**, 655–670 (1976).

² Vizard, D. L. & Ansevin, A. T. *Biochemistry* **15**, 741–750 (1976).

³ Lyubchenko, Yu. L., Frank-Kamenetskii, M. D., Vologodskii, A. V., Lazurkin, Yu. S. & Gauze, G. G., Jr *Biopolymers* **15**, 1019–1036 (1976).

⁴ Wada, A., Tachibana, H., Gotoh, O. & Takanami, M. *Nature* **263**, 439–440 (1976).

⁵ Akiyama, C., Gotoh, O. & Wada, A. *Biopolymers* **16**, 427–435 (1977).

⁶ Sanger, F. *et al.* *Nature* **265**, 687–695 (1977).

⁷ Machida, Y., Okazaki, T. & Okazaki, R. *Proc. natn. Acad. Sci. U.S.A.* (in the press).

⁸ Hayashi, M. N. & Hayashi, M. *J. Virol.* **14**, 1142–1152 (1974).

⁹ Takanami, M., Okamoto, T., Sugimoto, K. & Sugisaki, H. *J. molec. Biol.* **95**, 21–31 (1975).

matters arising

Generation length and rates of hominoid molecular evolution

BENVENISTE and Todaro¹ recently presented a large and valuable body of DNA annealing data bearing on Old World monkey, ape, and human evolutionary relationships. For an evolutionary biologist, the basic advantages of such data lie in their potential to provide phylogenetic information with minimal reference to other sources of comparative data on the organisms under study. That is, the molecular data set can, in large part, be tested internally and thus tell its own message. We therefore find it disturbing that Benveniste and Todaro have carried out a phylogenetic interpretation of their data using the assumption that generation length affects rates of nucleic acid evolution—especially when one notes that they present on the same page data from their own work which strongly suggest that it does not. We have specific reference to Fig. 1 and Table 2 of ref. 1.

In Fig. 1 of ref. 1 the authors calculate intrahominoid divergence times assuming an Old World monkey–ape time of 30 Myr and a rate of DNA base substitution inversely proportional to the estimated generation lengths involved. That is, longer generation length forms would undergo less rapid DNA evolutionary change. In this fashion, dates reasonably compatible with current palaeontological views of hominoid evolution² are obtained. Thus Benveniste and Todaro fail to note that the data of their Tables 1 and 2 show no such dependence on the proposed generation lengths.

We can reach this conclusion because the generation length hypothesis is, fortunately, subject to direct test. Whatever the divergence time of, for example, the human and gibbon lineages, both have obviously existed for the same length of time since their separation. If the generation length hypothesis were valid, then the human line would have accumulated fewer nucleotide substitutions in that time than the gibbon line. Therefore our DNA would today remain more similar to that of a monkey than is modern gibbon DNA. Specifically we note the human–gibbon $\Delta T_m R$ of 6.9 °C. In the legend to Fig. 1, Benveniste and Todaro state that they assume for the gibbon line a value of 1 °C $\Delta T_m R$ per 3 Myr of evolution, and for the human line 1 °C $\Delta T_m R$ per 5 Myr. Since the

Taxa compared	DNA*†	Albumin plus* Transferrin Immunology	Time of divergence (Myr)
<i>Homo–Pan–Gorilla</i>	0.14	0.12	4–5
(H,P,G)– <i>Pongo</i>	0.3	0.25	9–11
(H,P,G)– <i>Hylobates</i>	0.36	0.29	11–13
Apes, man–Old World monkeys	0.60	0.57	20–22
Catarrhini–Platyrrhini	1.0	1.0	35–38
Prosimii–Anthropoidea	—‡	2.0	70–75

*Each number in these columns is the ratio of the distance between those taxa and the catarrhine–platyrrhine distance.

†The first three numbers are means of the relevant data points in refs. 1, 3, and 9.

‡Prosimian–anthropoid differences are apparently too large to give reliable DNA data. But, our immunological studies of albumin and transferrin evolution in primates and other mammals show very similar amounts of change along the various primate lineages along with a prosimian–anthropoid distance almost exactly twice that between New and Old World higher primates (platyrrhines and catarrhines)¹⁰. Placing the beginnings of the adaptive radiation leading to modern primates at 70–75 Myr, this gives a platyrrhine–catarrhine separation at ~35 Myr (ref. 11).

human and gibbon lines separated, then, the latter would have accumulated 5/3 as many nucleotide substitutions. That is, of the 6.9 °C, 4.3 °C would have accumulated along the gibbon line, and 2.6 °C along the human. Thus when human and gibbon DNAs were compared to that of an Old World monkey (baboon), that of the gibbon should have given a $\Delta T_m R$ 1.7 °C larger than that given by human DNA, and such a difference is several times greater than the experimental errors implicit in such DNA comparisons. Yet precisely this comparison is presented in Tables 1 and 2 of ref. 1, where we note the baboon–human difference to be 9.1 °C, while the baboon–gibbon difference is 9.05 °C. Indeed, all five hominoids tested are in the range 9.15 ± 0.15 °C, that is identical within experimental error. As the actual data, by direct test, fail to show the assumed generation length effect, why allege its influence in time of divergence calculations? This is, after all, not a new issue. Other authors have argued for generation length effects in hominoid molecular evolution^{3,4}, and had their arguments answered^{5–8}. Indeed, Kohne³, though favouring the generation length hypothesis, had nevertheless allocated the same amounts of change along the human and gibbon DNA lineages as long ago as 1970.

In the absence of any documentation of a generation length effect, and with the direct demonstration that amounts of DNA change along the various primate lineages are a function of the temporal lengths of those lineages, we feel amply justified in using the available data to calculate divergence times as in the accompanying Table 1.

The particular virtue of protein and

nucleic acid data is that we can actually count interspecies differences in constant units and allocate them along lineages with minimal dependence on non-molecular evidence. Unfortunately, it has far too often been the case that the molecular data have been forced into a misguided congruence with what is thought to be hard evidence from a sparse and spotty primate fossil record—thereby long-delaying the day when those data will achieve their proper and invaluable place in providing that framework within which to most usefully view other bodies of evolutionary evidence, including those deriving from the fossil record.

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- 1 Benveniste, R. E. & Todaro, G. J. *Nature* **261**, 101–108 (1976).
- 2 Simons, E. L. *Primate Evolution* (Macmillan, New York, 1972).
- 3 Kohne, D. E. *Q. Rev. Biophys.* **3**, 327–375 (1970).
- 4 Lovejoy, O., Burstein, H. & Heiple, K. H. *Science* **176**, 803–806 (1972).
- 5 Sarich, V. M. *Biochem. Genet.* **7**, 205–212 (1972).
- 6 Sarich, V. M. & Wilson, A. C. *Science* **171**, 1144–1147 (1973).
- 7 Sarich, V. M. *Yb. Phys. Anthro.* **17**, 98–112 (1973).
- 8 Wilson, A. C., Carlson, S.S. and White, T. J. *A. Rev. Biochem.* **46**, 573–639 (1977).
- 9 Hoyer, B. H., van de Velde, N. W., Goodman, M. & Roberts, R. B. *J. hum. Evol.* **1**, 645–649 (1972).
- 10 Sarich, V. M. & Cronin, J. E. in *Molecular Anthropology* (eds Goodman, M. & Tashian, R. E.) 139–168 (Plenum, New York, 1977).
- 11 Cronin, J. E. & Sarich, V. M. *J. hum. Evol.* **4**, 357–375 (1975).

TODARO AND BENVENISTE REPLY—We are aware of the long-standing discussion in the literature concerning the effect of generation length of a species on the rate of accumulation of base-pair mutations

in cellular DNA (refs 1, 2) and amino acid substitutions in proteins^{3,4}. But, the main conclusions of our paper did not depend on knowing the proper corrections to apply, if any, for the lengthened generation times in the higher apes and man as compared to the Old World monkeys. The conclusions were that (1), nucleic acid sequences related to the genome of the genetically transmitted baboon type C virus can be detected in the cellular DNA of all Old World monkeys and apes; (2), the baboon type C virus specific sequences can be used to discriminate between those Old World monkeys and apes whose habitat is Africa (baboon, mangabey, African green monkey, colobus, chimpanzee, gorilla) and those whose habitat is Asia (various macaques, langurs, gibbon, orangutan); (3), the data obtained with human DNA closely fit the pattern observed with the Asian apes. The suggestion was therefore made that most of man's evolution since his divergence from the other apes had occurred in Asia rather than in Africa.

Sarich and Cronin point out that the hypothesis that animals with longer generation times accumulate fewer mutations per unit of chronological time as suggested by Kohne¹, Lovejoy⁵, and others is subject to a direct test. The accumulation of nucleotide substitutions along the gibbon lineage, where the generation length has changed less, compared to Old World monkeys, should be more than it is along the human lineage where the time of sexual maturity and the maximum lifetime potential⁶ have markedly increased. Although we agree with him that this is experimentally testable, the differences would be small. If man had a common ancestor with the chimpanzee and gorilla as recently as 4 Myr ago as Sarich has proposed^{7,8}, then the effect of the recent prolonged generation time of ancestral man would be even more difficult to detect. We would not conclude that the cellular DNA data as reported in the manuscript was performed in a sufficiently rigorous fashion to 'prove' the lack of effect of a generation time. In our initial studies we did not see a difference along the two lineages, but more refined studies directed specifically at that question might reveal small differences; such a study is currently in progress.

Our data concerning the relationships among the cellular DNA of Old World monkeys and apes agree quite well with the extensive immunological studies of Sarich, Cronin, and others. For example, we state that 'the extent of nucleic acid sequence divergence among chimpanzee, gorilla, and man is three to four times less than that between any of these three Homininae and the Old World monkeys'. But, there is also a considerable body of palaeontological evidence suggesting that apes and hominids had differentiated long before the time Sarich and associates

place the chimp-gorilla-man common ancestor^{6,9}. With methods for dating fossils improving, this is now a substantial body of data we felt should not be ignored. It will be intriguing to see if the traditional palaeontological approach can be correlated to the data being obtained with respect to protein and nucleic acid evolution, or whether the discrepancy in the two methodologies is as large as Sarich suggests.

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NIH Bethesda, M.D.

- ¹ Kohne, D. E. *Q. Rev. Biophys.* 3, 327-375 (1970).
- ² Kohne, D. E., Chiscon, J. A. & Hoyer, B. H. *J. hum. Evol.* 1, 627-644 (1972).
- ³ Goodman, M., Barnabas, J. & Moore, G. W. *J. hum. Evol.* 1, 663-686 (1972).
- ⁴ Goodman, M., Moore, G. W. & Matsuda, G. *Nature* 253, 603-608 (1975).
- ⁵ Lovejoy, C. O., Burnstein, A. H. & Heiple, K. G. *Science* 176, 803-805 (1972).
- ⁶ Cutler, R. G. *J. hum. Evol.* 5, 169-202 (1976).
- ⁷ Sarich, V. M. in *Perspectives on Human Evolution* (eds Washburn, S. L. & Jay, P. C.) 94 (Rinehart, Holt and Winston, New York, 1968).
- ⁸ Sarich, V. M. & Wilson, A. C. *Proc. natn. Acad. Sci. U.S.A.* 63, 1088-1091 (1969).
- ⁹ Simons, E. L. *J. hum. Evol.* 5, 511-528 (1976).

Complementation of immune response genes for (T,G)-A-L

Two years ago we reported the results of experiments which demonstrated the *in vivo* complementation of immune response genes required for a response to (T,G)-A-L (ref. 1). Subsequent experiments seemed to confirm and extend this result². Unfortunately others have been unable to repeat our results in as much as they find no evidence for complementation in the response to (T,G)-A-L (ref. 3). Recently in attempts to repeat our original findings with (B10.M×B10.Br)F₁ we also have been unable to do so but now find responses to (T,G)-A-L similar to those reported by others³. We wish, therefore, to express our reservations concerning complementation in the (B10.M×B10.Br)F₁, in the hope that further study of this phenomenon by ourselves and others will clarify the conditions in which genetic complementation in the response to (T,G)-A-L can occur.

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¹ Munro, A. J. & Taussig, M. J. *Nature* 256, 103-107 (1975).

² Munro, A. J., Taussig, M. J. & Archer, J. in *3rd Ir gene workshop* (ed. McDevitt, H. O.) (Academic, New York, in the press).

³ McDevitt, H. O. in *The Role of Products of the Histocompatibility Complex in Immune Responses* (eds Katz, D. H. & Benacerraf, B. A.) 321-326 (Academic, New York, 1976).

High explosive analogue of the Tunguska Event

JONES¹, observes the existence of an important, but little known experiment. The numerical conclusions however are incorrect, using only the

data cited in the letter. Using the ratio 35,000 to 130 as the diameters of symmetric explosion phenomena for the Tunguska event and the 1966 experiment cited by Jones, respectively, and the relevant scaling law, the corresponding equivalent explosive source strength for the Tunguska event would appear to be of the order of $50 \times (35,000/130)^3$, or about 10^9 t, not the 10^5 t cited. Without accurately computing all the necessary corrections for energy partitioning, actual hydrodynamics, etc., this would provide an estimate of source strength about two orders of magnitude higher than ref. 3 cited therein concludes.

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¹ Jones, G. H. S. *Nature* 267, 605 (1977).

JONES replies: Augenstein is correct, and the calculated values should have read 10^9 kt TNT and 200 Mt nuclear equivalent, these being minimum estimates. The Tunguska event devastated an area 35 km in diameter! It is commented, however, that the very low height of burst, almost a surface burst at this yield, should be retained despite the absence of a crater at Tunguska. I have discussed elsewhere (Suffield Reports 217 and 281), experimental evidence that high yield but low energy density explosions on the surface do not cause craters but do produce airblast and surface seismic effects similar to those from high density explosions. Forest blowdown, for example, is insensitive to the initial energy density but depends upon the total energy release. The lack of a crater at Tunguska is thus evidence of the low density, presumably cometary nature of the bolide.

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β-Globin mRNA in Ferrara β⁰-thalassaemia

In their paper on the presence of β-mRNA in Ferrara type β⁰-thalassaemia, Ottolenghi *et al.*¹ stated that our laboratory had obtained "similar findings" to their's with material from one of their patients. Because of possible misinterpretations of that very general statement, we present here the limited data which we did obtain using Ferrara material.

Professor Conconi kindly provided us with ribosomes from one of his patients so that we could attempt to

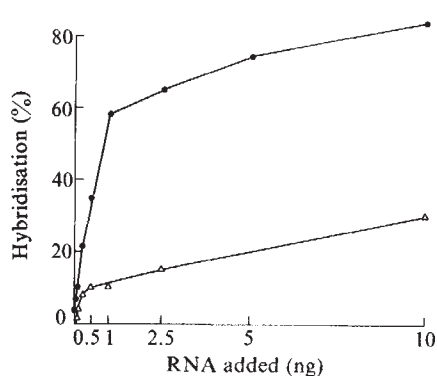


Fig. 1 Hybridisation of Ferrara β^0 -thalassaemia RNA to human α - and β -cDNA. ●, α -DNA; △, β -cDNA.

demonstrate β -globin mRNA, in RNA extracted from these ribosomes, by fingerprint analysis of ^{32}P -labelled cRNA synthesised from cDNA²; cDNA was obtained but the cRNA synthesis failed. We did, however, use a small amount of the starting RNA to assay its relative content of α and β mRNA by molecular hybridisation to human α and β globin cDNA as previously described³. A small amount of β or β -like mRNA was detected (Fig. 1). Unfortunately there was insufficient RNA available to obtain a plateau of hybridisation with β -cDNA but the slope of the β -cDNA hybridisation curve predicts a β -mRNA content in the sample of approximately 4% relative to that of the α mRNA in the same sample. Because we lacked sufficient material to demonstrate a plateau or saturation of hybridisation of Ferrara β -like mRNA with β -cDNA, our results do not help to resolve the discrepancy between the results of Ottolenghi *et al.*¹ and those of Ramirez *et al.*⁴. The latter found that the Ferrara β -like mRNA hybridised incompletely with β cDNA (in contrast to complete hybridisation claimed by Ottolenghi *et al.*) and suggested the presence of a substantial abnormality in the structure of Ferrara β mRNA.

Our hybridisation results with Ferrara thalassaemia mRNA are similar to those which we have obtained⁵ with total reticulocyte RNA from a variety of β^0 -thalassaemic patients of different ethnic backgrounds (Southern Italian, Greek, Algerian, Asian and Chinese). In some of these patients there is less than 1% as much β -like mRNA as α mRNA⁶ and this could be due to cross hybridisation of δ -chain mRNA of HbA₂ to β cDNA. In approximately half the patients, however⁵, we have found 4–15% as much β -like mRNA as α mRNA; the latter value is probably too high to be accounted for by δ -chain mRNA alone. Other workers have found substantially more β -like mRNA

in non-Ferrara β^0 thalassaemic reticulocytes^{4,7}. We were unable to identify, by fingerprint analysis of ^{125}I -labelled mRNA, β -chain specific oligonucleotides in RNA from two of our patients with low levels of β -like mRNA detected by hybridisation⁵. But, Temple *et al.*⁸ have been successful in detecting β -chain specific mRNA sequences in one of their patients with β^0 -thalassaemia.

We suggest that until specific structural differences in the β -globin gene of β^0 -thalassaemics are detected by gene mapping or nucleotide sequence studies, one cannot make any definitive conclusions as to the precise molecular basis of the varied forms of β^0 -thalassaemia. The β -globin genes seem to be present in all cases of β^0 -thalassaemia in which they have been sought (refs 4, 7–9 and Tuan, D.G.H. and B.G.F., unpublished). The different results obtained by hybridisation assays of globin mRNA need to be related to specific structural defects in the β -globin gene DNA before one can feel confident about classifying the β^0 -thalassaemias found in different populations into specific molecular subtypes.

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² Forget, B. G. *et al.* *Ann. N.Y. Acad. Sci.* **241**, 290–309 (1974).

³ Forget, B. G. *et al.* *Cell* **7**, 323–329 (1976).

⁴ Ramirez, F. *et al.* *Nature* **263**, 471–475 (1976).

⁵ Forget, B. G., Hillman, D. G., Cohen-Solal, M. & Prensley, W. *Blood* **48**, 998 (1976).

⁶ Forget, B. G., Benz, E. J., Jr., Skoultschi, A., Baglioni, C. & Housman, D. *Nature* **247**, 379–381 (1974).

⁷ Kan, Y. W., Holland, J. P., Dozy, A. M. & Varmus, H. E. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5140–5144 (1975).

⁸ Temple, G. F., Chang, J. & Kan, Y. W. *Proc. natn. Acad. Sci. U.S.A.* **74**, 3047–3051 (1977).

⁹ Tolstoshev, P. *et al.* *Nature* **259**, 95–98 (1976).

OTTOLENGHI ET AL. REPLY—Forget and Hillman conclude that the different results obtained by hybridisation assay of globin mRNA by various groups^{1–5} do not yet allow the classification of β^0 -thalassaemia found in different populations into specific molecular subtypes; this conclusion is based on the wide range of values reported for β -globin-like RNA in non-Ferrara β^0 -thalassaemic patients, and the overlapping of some of these values with those determined by ourselves⁵ and by Forget and Hillman in Ferrara β^0 -patients. As has been pointed out by Benz^{6,7}, however, discrepancies in the level of β -like sequences found in β^0 -thalassaemias may arise as the result of technical differences between laboratories.

In our laboratory we have compared

several different groups of patients. β -like mRNA has been either undetectable in non-Ferrara β^0 -thalassaemic reticulocytes⁸ or present at levels of less than 1% relative to α -sequences (a value that is compatible with cross-hybridisation between cDNA _{β} and mRNA _{δ} sequences), whereas in reticulocytes of Ferrara β^0 -thalassaemics levels of up to 8% can be detected. Moreover, when sufficient RNA has been available, the T_m of the hybrid formed between the low amounts of ' β -like RNA' from non-Ferrara β^0 -thalassaemics (? δ -mRNA) and cDNA _{β} has been 7–8 °C lower than that found for true β -mRNA–cDNA hybrids⁸, again in significant contrast with what we find in Ferrara thalassaemia.

We, therefore, conclude that our results (refs 5, 9 and unpublished) taken together with the translational data of Conconi's group^{10,11} allow us to divide β^0 -thalassaemia into at least two different types, a β -mRNA negative type and a β -mRNA positive type; whether other forms of β^0 -thalassaemia in which significant levels of β -mRNA have been demonstrated have any relationship with the Ferrara type of thalassaemia remains to be investigated, and we agree will require translational experiments, as well as gene mapping and nucleotide sequence studies.

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² Kan, Y. W., Holland, J. P., Dozy, A. M. & Varmus, H. E. *Proc. natn. Acad. Sci. U.S.A.* **72**, 5140–5144 (1975).

³ Forget, B. G., Hillman, D. G., Cohen-Solal, M. & Prensley, W. *Blood* **48**, 998 (1976).

⁴ Ramirez, F. *et al.* *Nature* **263**, 471–475 (1976).

⁵ Ottolenghi, S. *et al.* *Nature* **266**, 231–234 (1977).

⁶ Benz, E. J., Jr. *Nature* **263**, 635–636 (1976).

⁷ Benz, E. J., Jr. *et al.* *Clin. Res.* **23**, 269a (1975).

⁸ Comi, P. *et al.* *Eur. J. Biochem.* (in the press).

⁹ Tolstoshev, P. *et al.* *Nature* **259**, 95–98 (1976).

¹⁰ Conconi, F., Rowley, P. T., del Senno, L., Pontremoli, S. & Volpato, S. *Nature new Biol.* **238**, 83–87 (1972).

¹¹ Conconi, F. *et al.* *Nature* **254**, 256–259 (1975).

reviews

Introducing relativity

Paul Davies

Essential Relativity: Special, General and Cosmological. Second edition. By W. Rindler. Pp. xv+284. (Springer: New York and Berlin, 1977.) DM67.70; \$29.80.

AFTER eight years I still find Rindler's book one of the best introductions to the theory of relativity, and I was eager to read his second edition. The title *Essential Relativity* is an apt one, for even though this edition contains some new material, only the bare essentials of the subject are discussed. This is not a drawback, because Rindler manages somehow to get very far with very little investment in detailed calculation or protracted conceptual deliberation. In this respect it makes ideal introductory reading for students of physics, although mathematics students will not find much in the way of modern differential geometry or topology.

In the preface to the second edition the author explains how the emphasis of the book is on the conceptual foundations rather than the mathematical development of relativity theory. Proofs are kept to a minimum and many examples and mathematically-oriented discussion are only punctuated by, rather than rooted in, hard equations. Nevertheless, the reader is taken swiftly and effortlessly (maybe somewhat deceptively) through pre-Einstein ideas of space and time, basic special relativity, Minkowski space and four-vectors, tensors, particle mechanics and electrodynamics. Then we move into general relativity, which starts out with the most 'conceptual' section of the book, in which the idea of curved space-time is explained in just a few pages. The Schwarzschild metric just seems to pop out from this discussion, to be followed by an updated description of black holes and the Kuskal manifold.

When I re-read the section on uniformly accelerated observers in Minkowski space, I was disappointed to find that the author, whose name is famous for his connection with the co-ordination of Minkowski space based on a collection of accelerating observers, had not chosen to expand more on this very useful construction. I have found the ideas of uniform acceleration both useful and elegant exercises for undergraduates, as well as

a very profitable analogy for the more advanced discussions of black holes.

The book concludes with a section on cosmology, and once again I was interested to see if Rindler's discussion of horizons had been elaborated. The earlier edition was one of the first undergraduate textbooks of its time to try and present this subject conceptually. The new edition contains the familiar remarks about crawling bugs and rubber sheets, as well as the usual mathematical conditions for horizons, but also includes some new material and diagrams to help untangle some of the apparent paradoxes associated with them.

Although the organisation of the material is largely the same as before, many sections have been re-written, in the same lucid style. New sections on the plane gravitational wave and linearised general relativity, as well as two appendices on curvature and Maxwell theory, have been added. I was disappointed at the price for a book which should be aiming at the 'mass' student market, to which it is admirably suited. How many undergraduates will pay nearly £20 for a basic course textbook? □

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Immunological series

Comprehensive Immunology. Vol. 1: Immunology and Aging. Edited by T. Makinodan and E. Yunis. Pp. 208. Vol. 2: Biological Amplification Systems in Immunology. Edited by N. K. Day and R. A. Good. Pp. 325. (Plenum: New York and London, 1977.) \$27 each volume.

THE new series *Comprehensive Immunology* is intended eventually to cover all aspects of immunology with "definitive analyses, thoughtful reviews and probing discussions". There already exist several good series on immunological subjects and the launching of another cannot be greeted with unmixed joy. These first two volumes, however, are well produced and have some valuable features. If their standard can be maintained, the series should prove useful.

The appearance of *Immunology and Aging* is timely, since no comprehensive review has been available and increasing interest is being shown in the field. It is also a good moment to look back over the large amount of data that have accumulated on ageing in relation to the immune system. We are still far from understanding this relationship, and it is certainly complex. The thymus and T lymphocytes, however, are widely believed to be among the central characters in the drama, and several papers emphasise this from various points of view. The known intricacies of the membrane structure and physiological functions of T lymphocytes have

been multiplying so rapidly of late that T-dependent interpretations of gerontological phenomena tend to age rather quickly; but this problem should be resolved within the next few years. Among other good chapters are two dealing with germ-free mice and congenitally athymic (nude) mice and their value in the study of ageing. Overall, the volume can be recommended to anyone seeking an introduction to both biological and clinical aspects of the subject, and the extensive reference lists make it an excellent source book.

I tried the title *Biological Amplification Systems in Immunology* on several immunological colleagues, inviting speculation on the contents of this volume. They produced a range of suggestions, none of which approximated to the truth. I can now reveal exclusively to readers of *Nature* that the book is about complement. Probably an expert on complement would have tumbled to it instantly, but then I don't think that the book is really intended for him. The 15 chapters cover the multiple roles of the complement system, as well as its biosynthesis, phylogeny, ontogeny and genetics. There are also discussions of leukocyte chemotaxis and of pathways activated by Hageman Factor. It seems a pity that this useful collection of reviews should be hidden under such an enigmatic title. **H. S. Micklem**

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Unparalleled textbook of plant anatomy

Anatomy of Seed Plants. Second edition. Pp. 550. By Katherine Esau. (Wiley: London and New York, 1977.) \$21.25; £11.50.

TWENTY FOUR YEARS AGO, Professor Katherine Esau published her first book, *Plant Anatomy*. This book rapidly established itself as a standard by which all others have subsequently been judged. As Professor Esau herself points out, however, there was also a need for a shorter book for students who have relatively limited experience in the study of plants. For that reason *Anatomy of Seed Plants* was first published in 1960 and is now seen in its second edition.

Anatomy of Seed Plants still has to be viewed against the broader, and deeper, backcloth set by *Plant Anatomy* itself, but it remains an attractive, informative and authoritative book in its own right, even if its price will restrict ownership more severely than one might hope. An important feature of this second edition is that it incorporates very significant changes and is very much more than a new edition in name alone.

An immediately apparent change is in the page format which is now enlarged to 23.3 cm × 18.9 cm from the previous 22.8 cm × 14.9 cm—a 30% increase in area. This, together with the fact that the book is extended from 376 to 550 pages, has allowed incorporation of much new material, although the larger page size has often been wasted where micrographs and diagrams from the first edition have been reproduced at the same size. The pleasant format, together with two-column presentation, a clearly legible type-face and a balanced distribution of text and illustrations, has led to the production of a book that demands to be read.

Having attracted the reader's attention, what is to be found inside? A major, and immediately obvious feature is the inclusion of numerous electron micrographs. There were only a couple in the first edition, but the present book is liberally laced with them and they are, as are most of the other illustrations chosen, of good quality. If anything, the half-tones in the present volume are of better contrast than those in the original.

Professor Esau's attention to detail is prodigious and only the closest scrutiny of the book reveals many of the smaller changes that have been

made to take account of advances in knowledge or interpretation over the past 16 years. (The abbreviation for micrometre is now given in its proper form (μm) but it is a pity that the Ångström lingers on.) The enormous task of encompassing new information without completely losing sight of the old has largely been achieved by combining subject matter as much as possible, and by referring only to key items of interest within a particular area of study. Similarly, the references, though strictly limited, serve to acquaint the reader with the most recent work and generally include at least one paper from each main laboratory contributing to a specific field of interest. In this way, *Anatomy of Seed Plants* is able to cover a large subject area while allowing the reader

the opportunity to find his way more deeply into plant anatomy if he so desires.

Anatomy of Seed Plants is unashamedly a textbook of anatomy. As such it is unparalleled. Although the modern trend is, and must be, to combine structure and function in our approach to the way plants work, this can only be accomplished if our basic knowledge of plant anatomy is both broad and sound. Professor Esau has ensured that no botanist—whether student or research worker—will have the excuse that the right information is not readily, and attractively, to hand.

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Diatom biology

Biology of Diatoms. Edited by Dietrich Werner. Pp. 498. (Blackwell Scientific: Oxford, 1977.) £17.

Few algal groups have seen such an expansion of interest over the past two decades as the diatoms, and this volume admirably covers the recent developments. It may come as a surprise to many to read on the first page that it is estimated that diatoms contribute 20–25% of the world's net primary production—a figure derived from experimental studies which almost certainly underestimate true production. Such production at the base of so many food chains ought to stimulate work on grazing, yet this is hardly begun and is mentioned on only a few pages.

Although the title might imply that this book can be read to obtain an overall survey of the diatoms, this is not so, and a student with little knowledge of the group would find it difficult. There is no section on morphology, no simple explanation of cell division, few illustrations of the major groups; and those given are of centric genera leaving a student to guess at pennate morphology.

Growth, nutrition, chemical composition, movement, sexuality, ecology are all discussed; in these chapters, each written by a different author, there is a wealth of data and excellent references. The authors of the chapter on the ecology of marine planktonic diatoms have assembled a mass of fascinating data on distribution in the seas of the world which is a valuable contribution to the much neglected topic of biogeography.

In many places, however, the text is clearly written in review form and

many statements merely stimulate and necessitate consultation of the original papers. The chapter on sexuality will be of great value to many students since much of the excellent original work is very discursive and written in German.

A chapter on ultrastructure reviews the literature but is very poorly illustrated with three very indistinct pictures, all of *Cylindrotheca* which has an unusual morphology. This is a pity since there are now several excellent studies of diatom ultrastructure. Also, apart from a few photographs of centric diatoms, the considerable body of information on the ultrastructure of the siliceous components has not been utilised. The old concept of diatoms as a class of the Chrysophyta again crops up in some chapters although they were clearly recognised as a division, Bacillariophyta, in the system of Hustedt (1930) and are so distinctive that there can be surely little argument about this in 1977.

Biochemical and physiological studies have blossomed in the past two decades, and the information in the chapters on growth, silicate metabolism, photosynthesis, heterotrophic nutrition and biochemical composition will surprise many who have not followed the field closely. The chapter on silicate metabolism is of great value, since it is a synthesis of chemical and biological studies and provides a valuable introduction to the complexities of silicon chemistry.

The combination of authors has resulted in some repetition of data in several chapters but not in any serious way. The book is well produced, relatively free of errors and will be a source of considerable value for many years to come.

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Physiological behaviour of cells

Analytical Physiology of Cells and Developing Organisms. By B. C. Goodwin. Pp. 249. (Academic: London and New York, 1977.) £8.50; \$18.50.

THE aim of this book is to present a picture of the physiological behaviour of cells in terms of molecular biology and mathematical models. As the author acknowledges, the choice of material is not comprehensive and reflects his own interests and prejudices. In the first three chapters, Goodwin examines aspects of the behaviour of individual cells. The metabolic network, the adaptation time of which is of the order of seconds, is considered in terms of allosteric enzymes and end-product inhibition. This network is contrasted with the epigenetic one with longer adaptation times (minutes to hours) and in which gene activation and protein synthesis are now major elements. The occurrence of feedback loops in both kinds of systems can lead to complex dynamics such as oscillations. Analogies with statistical thermodynamics are drawn; and this rather formal analysis is extended to the cell cycle.

In considering the various internal networks involved, the important point is made that there may be no single reaction controlling the entry of a cell into the cell cycle: emphasis should be placed instead on a mixture of probabilistic and deterministic processes. The treatment of the cell cycle is, however, unsatisfactory since it provides an inadequate review of current ideas and experiments. For example, although mechanisms based on chaperones which are inhibitory are considered, there is little reference to molecules exerting a positive control, for which there is increasing evidence. A nice chapter on biological clocks follows, with an explanation of Winfree's ingenious approach to the eclosion clocks in *Drosophila*. Here, mathematical analysis leads to experiments that could not really be conceived of without that analysis. Unfortunately, most of the other analyses in the book do not seem to have a similar experimental spin-off.

Pattern formation and its relation to gradients and positional information is given quite detailed attention. Emphasis is placed on periodic processes and a membrane wave model is put forward. A formal model to account for the properties of the polar coordinate model of French, Bryant and Bryant is proposed. Goodwin believes in the next

few years we will see the emergence of new dynamical models in which waves and periodicities will feature very strongly. He recognises that gradient-type models of positional information shift the major problem of pattern formation into the area of how the gradients are interpreted by the cells in terms of gene activity, but gives this central theoretical problem inadequate attention.

In the final chapter, he treats the organism as a cognitive and cooperative system. He treats the cell as if it had knowledge about the world which is contained in its control circuits. He also wishes to regard sharp transitions of state at different levels of organisation as being basically similar and analogous to phase transitions.

Goodwin is always worth reading for his independence and insights, but overall this book is disappointing. The mathematics seems to add rather little and too seldom leads to experiments. The approach is not didactic or comprehensive. For theoreticians, however, it is essential.

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Quantum theory

Qualitative Methods in Quantum Theory. By A. B. Migdal. Pp. 435. (W. A. Benjamin: Reading, Massachusetts and London, 1977.) \$21.50.

"THE solution of most problems in theoretical physics begins with the application of the *qualitative methods* which constitute the most attractive and beautiful characteristic of this discipline. By 'qualitative methods' we mean dimensional estimates and estimates made by using simple models, the investigation of limiting cases where one can exploit the smallness of some parameter, the use of the analytic properties of physical quantities, and finally the derivation of consequences from the symmetry properties, that is, the invariance relative to various transformations (eg Lorentz or isotopic invariance). However, as experience in the classroom shows, it is just these aspects of theoretical physics which are most difficult for the beginner." So Academician Migdal introduces the new edition of his book, the first three chapters of which were published in much the same form as

in *Approximation Methods in Quantum Mechanics* (Izdatelstvo Nauka, 1966; English translation, W. A. Benjamin, 1969).

Migdal is a leading exponent of the Landau school of theoretical physics, the members of which have been brought up to know all nine volumes of Landau and Lifshitz. So in this book we have examples from a wide range of subjects. The first chapter covers mainly models in atomic physics. Chapter two is on perturbation theory, and chapter three is on the WKB method and the quasi-classical approximation, a subject which is not normally given much space in textbooks. Chapter four is on analyticity and dispersion relations. Chapter five is on quasi-particle methods in the many-body problem, with applications to quantum fluids and nuclear matter. Chapter six gives a qualitative physical treatment of renormalisation, and a discussion of the Gell-Mann-Low equation and asymptotic freedom.

This book should be required reading for any serious graduate student in theoretical physics.

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Renaissance in laser spectroscopy

Frontiers in Laser Spectroscopy. (Les Houches 1975 Session XXVII) Vol. 1. Pp. 472. Vol. 2. Pp. 473–907. Edited by Roger Balian, Serge Haroche and Sylvain Liberman. (North-Holland: Amsterdam, New York and Oxford, 1977.) Dfl. 280; \$114.50.

THESE two volumes present the proceedings of the Les Houches Theoretical Physics Summer School held in July 1975 on the use of lasers in atomic and molecular spectroscopy. The stated aim of the school was to "give a general presentation of the theoretical background necessary to understand light-matter interaction problems, and to present a review of the various new methods in which lasers are now being used to improve our knowledge about atoms, molecules and even about nuclei." Underlying these new methods are the recent exploitation of tunability, of saturation and of coherent transients which have contributed so much of late to the renaissance in spectroscopy. The level of presentation of the proceedings tends to assume previous knowledge at about first-year graduate student level of quantum optics and quantum electronics, and the scope of the discussion is very wide with most of the actively-pursued topics in the field at least mentioned.

Cohen-Tannoudji presents a marginally telegraphic overview of the basic phenomena involved in the interaction of atoms with strong resonant fields, and his thoughtful lectures contain many insights into fundamental problems involving the non-linear saturation of fluorescent emission. This is followed in Volume 1 by lectures on laser theory (Sargent); transient and steady-state response of two- and three-level systems, including a very useful discussion of the semiclassical theory of superradiance (Feld); the theory and observation by stark-switching of optical nutation, free-induction decay, photon echoes and two-photon transitions (Brewer); and single-atom and co-operative quantum beats (Stenholm). These lectures form a coherent and well-presented review of a physically well-defined area.

In Volume 2, this coherence is lost to a certain extent by an attempt to cover a very wide range of techniques and specific applications. The use of lasers in very high resolution spectroscopy of molecules is discussed by Lehmann, Oka, Kelley and Javan. Letokhov discusses the coupling be-

tween electronic and nuclear degrees of freedom in laser-driven transitions and the so-far purely theoretical subject of "laser-nuclear spectroscopy."

Finally, one of the topics which ensures adequate funding, namely isotope separation, is admirably discussed by Letokhov. Also included in these volumes are short seminars by Series, Walther, Cagnac, Haroche, Liberman, Toschek, Mukamel and Monchalin, which are useful as illustrations of particular research problems, although a few should have been (and some have been) published in the primary literature.

Some of these lectures are rather experimental in tone; these though no less valuable, do not quite fit the label of "Ecole d'Été de Physique Théorique." Indeed, an interesting feature

of this field is the ability of the best practitioners (some of them contributing to these volumes) to cross the experimental-theoretical divide to the great benefit of both sides. Since there were only 42 "pupils" attending the school, one would hope that the publication of the proceedings would give a wider circulation to these valuable lectures. The price of these volumes seems designed to ensure that this will not be so. I doubt the value of such lavish publication of conference and Summer school proceedings in hard-back textbook format, with its concomitant delay and inflated cost.

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Explosion in myelin research

Myelin. Edited by P. Morell. Pp. xxii+531. (Plenum: New York and London, 1977.) \$47.40.

DURING the past decade there has been an explosive increase in research on myelin. Now with the publication of a major book on the subject, it is possible to see why. Myelin is one of the most abundant of biological membranes constituting about 50% of the dry weight of the white matter, from which relatively pure fractions can be easily isolated by centrifugation. Such procedures are described in the book together with tabulated data of lipid and protein analysis of myelin fractions of central and peripheral nerve. Extensive biophysical data (for example, X-ray and neutron diffraction) has been correlated with the chemistry and the ingenious use of probe analysis. As a result, we probably know more about the molecular architecture of myelin than many other biological membranes.

The first chapter contains a great deal of impressive and well illustrated information on the morphology of the myelin-formative glial or Schwann cells. Although little is known about the mechanisms controlling the transition stage between glial plasma membrane and the fully mature compact myelin lamellae, the glial cell must, during the period of myelination, have a remarkable synthetic activity, for it seems to be capable of making three times its weight of myelin per day. The myelinating brain therefore provides a unique system for studying membrane biosynthesis. Some of the relevant precursor-product labelling

studies and the so far equivocal attempts at isolation of intermediary 'myelin-like' fractions from developing brain are critically discussed by different contributors.

Study of the biology of myelin is, however, of more than academic interest for in several neurological diseases there is damage to the white matter with accompanying loss of myelin. In the otherwise excellent chapters on neuropathology and neurology of myelin diseases more space could have usefully been devoted to multiple sclerosis, since this is one of the commonest neurological diseases.

Other chapters cover chemical pathology, experimental pathology and animal models of genetic myelin disorder. One of the most important features of myelin pathology relates to the pronounced immunological response to the protein antigens and lipid haptens (for example, cerebroside). This subject is especially relevant following the recent discovery of a relaxing and remitting form of experimental allergic encephalitis.

It will be evident that the whole intriguing subject of the biology of myelin is well covered in this book and it can be recommended as a readable and authoritative account of the subject.

Although the book is on a specialist subject it can be recommended to the general reader who might thereby be encouraged to utilise some of the basic concepts and discoveries on myelin in other fields of biology.

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Recent scientific and technical books

Mathematics

- ALCOCK, Donald. Illustrating Basic: (A Simple Programming Language). Pp.ix + 134. ISBN-0-521-21703-2. (Cambridge, London and New York: Cambridge University Press, 1977.) Hard cover £4.95; Limp cover £1.90.
- CAMERON, Peter J. (edited by). Combinatorial Surveys: Proceedings of the Sixth British Combinatorial Conference, Merton College, Oxford, England. Pp.vii + 226. ISBN-0-12-157150-5. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £7; \$13.65.
- JACOBSON, David H. Extensions of Linear-Quadratic Control, Optimization and Matrix Theory. (Mathematics in Science and Engineering: A Series of Monographs and Textbooks, Vol. 133.) Pp.x + 217. ISBN-0-12-378750-5. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £6.50; \$12.75.
- LAKESHMIKANTHAM, V. (edited by). Nonlinear Systems and Applications: An International Conference. Pp.xvi + 700. ISBN-0-12-434150-0. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$25; £17.75.
- NAGATA, Masayoshi. Field Theory: (Pure and Applied Mathematics: A Program of Monographs, Textbooks, and Lecture Notes: Vol. 40). Pp.vii + 268. ISBN-0-8247-6466-8 (New York and Basel: Marcel Dekker, 1977) \$Fr.78.
- PORTER, Richard D. Introduction to Fibre Bundles (Lecture Notes in Pure and Applied Mathematics, Vol. 31). Pp.v + 170. ISBN-0-8247-6626-1. (New York and Basel: Marcel Dekker, Inc., 1977.) \$Fr.65.

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- COLLOQUE DE L'UNION ASTRONOMIQUE INTERNATIONALE. No. 37: Décalages vers le Rouge et Expansion de l'Univers, Paris, 6-7 Septembre 1976, et Colloque International du Centre National de la Recherche Scientifique, No. 263: l'Evolution des Galaxies et ses Implications Cosmologiques, Paris, 8-9 Septembre 1976. Pp.619. ISBN-2-222-02022-0. (Paris: Editions du Centre National de la Recherche Scientifique, 1977.) 180 francs.
- HOYLE, Fred. Ten Faces of the Universe. Pp.ix + 207. ISBN-0-435-54427-6. (London: Heinemann Educational Books, Ltd., 1977.) £4.80 net.
- MOORE, Patrick, and COLLINS, Pete. The Astronomy of Southern Africa. Pp.160. ISBN-0-7091-676-X. (London: Robert Hale Limited, 1977.) £4.95.
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Chemistry

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Medicinals Group of the Industrial Division, The Chemical Society, and the Pesticides Group of the Society of Chemical Industry, Bangor, Wales, 15-17 September, 1976.) Pp.xi + 141. ISBN-0-85186-178-4. (London: The Chemical Society, 1977.) £7.50; \$15.

MCKILLOP, A. (senior reporter). Aliphatic Chemistry, Vol. 5. (A Review of the Literature published during 1975. A Specialist Periodical Report.) Pp.xi + 337. ISBN-0-85186-602-6. (London: The Chemical Society, 1977. Obtainable from The Chemical Society, Blackhorse Road, Letchworth, Herts.; American Chemical Society, 1155 Sixteenth Street, N.W., Washington, DC.) £23; \$47.

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- NAGL, Walter. Zellkern und Zellzyklen: Molekularbiologie, Organisation und Entwicklungsphysiologie der Desoxyribonucleinsäure und des Chromatins. Pp.486. ISBN-3-8001-3417-9. (Stuttgart: Verlag Eugen Ulmer, 1976.) DM 120.
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- WRIGHT, Sewall. Evolution and the Genetics of Populations. Vol. 3: Experimental Results and Evolutionary Deductions. Pp.613. ISBN-0-226-91051-2. (Chicago and London: The University of Chicago Press, 1977.) £24.50.
- ZUCKERMAN, Professor Lord, and WEIR, Barbara J. (edited by). The Ovary.

Vol. 1: General Aspects. Second edition. Pp.xviii+517. ISBN-0-12-782601-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$35; £24.85.

Applied Biology

- BHATTA, Balraj, CHHINA, G. S., and SINGH, Baldev. Selected Topics in Environmental Biology. (Based on the Sessions on Environmental Biology, held at the 26th International Congress on Physiological Sciences, New Delhi, October 20-26, 1974.) Pp.529. ISBN-0-08-021210-7. (Oxford and New York: Pergamon Press, 1977.) £66.50.
- BOREK, Carmia, FENOGLIO, Cecilia M., and KING, Donald West. (edited by). Cancer Biology, IV: Differentiation and Carcinogenesis. (Advances in Pathobiology, Vol. 6) Pp.xvi+323. ISBN-0-913258-48-2. (New York: Stratton Intercontinental Medical Book Corporation, 1977.) \$24.50.
- BRAIN, Joseph D., PROCTOR, Donald F., and REID, Lynne M. (edited by). Respiratory Defense Mechanisms, Part 1. (Lung Biology in Health and Disease, Vol. 5) Pp.xviii+488. ISBN-0-8247-6381-5. (New York and Basel: Marcel Dekker, Inc., 1977.) \$44.50.
- BUCKLEY, Joseph P., and FERRARIO, Carlos M. (edited by). In association with LOKHANDWALA, Mustafa, F. Central Actions of Angiotensin and Related Hormones. Pp.xvii+606. ISBN-0-08-020933-5. (Oxford and New York: Pergamon Press, 1977.) £27.
- BULLOCK, Theodore Holmes. With the collaboration of ORKAND, Richard, and GRINNELL, Alan. Introduction to Nervous Systems. (A Series of Books in Biology.) Pp.xiv+559. ISBN-0-7167-0577-X. (San Francisco and Reading: W. H. Freeman and Company, 1977.) £21.60.
- CHANG, Thomas Ming Swi. (edited by). Biomedical Applications of Immobilized Enzymes and Proteins. Vol. 1: Pp.xx+428. ISBN-0-306-34311-8. \$44.50 Vol. 2: Pp.xx+359. ISBN-0-306-34312-6 (v. 2). \$47.40. (New York and London: Plenum Press, 1977.)
- COAKER, T. H. (edited by) Applied Biology, Vol. 2. Pp.x+272. ISBN-0-12-040902-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £9.80; \$19.25. (Pub. 1 Aug. '77).
- DAUSETT and SVEJGAARD. (edited by). HLA and Disease. Pp.316. ISBN-X-87-16-02287-4. (Copenhagen: Munksgaard, North and South America: Baltimore: William & Wilkins Co., 1977.) D.kr. 180.00.
- DICKINSON, C. H., and LUCAS, J. A., Plant Pathology and Plant Pathogens. Vol. 6 (Basic Microbiology, edited by J. F. Wilkinson). Pp.x+161. ISBN-0-030399-5. (Oxford, London, Edinburgh and Melbourne: Blackwell Scientific Publications, 1977.) £4.25.
- GARDNER, Howard. The Shattered Mind: The Person After Brain Damage. Pp.xiv+481+viii. ISBN-0-7100-8641-5. (London and Henley: Routledge and Kegan Paul, Ltd., 1977.) £9.75.
- GEMMELL, RAYMOND, P., Colonization of Industrial Wasteland. (Studies in Biology no. 00). Pp.75. ISBN-0-7131-2587-X. (London; Edward Arnold (publishers) Ltd, 1977.) £3.20 (hardback) £1.60 (paperback).
- GUDZINOWICZ, Benjamin J., and GUDZINOWICZ, Michael J. With the assistance of MARTIN, Horace, and DRISCOLL, James L. Analysis of Drugs and Metabolites by Gas Chromatography-Mass Spectrometry. Vol. 1: Respiratory Gases, Volatile Anesthetics, Ethyl Alcohol, and Related Toxicological Materials. Pp.vii+238. ISBN-0-8247-6576-1. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr. 73.
- HILLS, Hilda Cherry. Good Food: Grain-Free, Milk-Free. Pp.123. (London: Roberts Publications, 225 Putney Bridge Road, SW15, 1977.) £2.25.
- HUGO, W. B., and RUSSELL, A. D., (edited by). Pharmaceutical Microbiology. Pp.vii+352. ISBN-0-632-00499-1. (Oxford: Blackwell Scientific Publications; 1977.) £8.50.
- JARRETT, A. (edited by). The Physiology and Pathophysiology of the Skin. Vol. 4: The Hair Follicle. Pp.xix+1237-1540+xxi. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £18.50; \$36.10.
- KELLY, Sally. Biochemical Methods in Medical Genetics. (A Monograph in The Bannerstone Division of American Lectures in Laboratory Medicine.) Pp.xi+345. ISBN-0-398-03630-6. (Springfield, Ill.: Charles C. Thomas, Publisher, 1977.) \$17.50.
- LANDSBERG, J. J., and CUTTING, C. V. (edited by). Environmental Effects on Crop Physiology. (Proceedings of a Symposium held at Long Ashton Research Station, University of Bristol, 13-16 April 1975. Fifth Long Ashton Symposium.) Pp.xv+388. ISBN-0-12-435050-X. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £14.50; \$28.35.
- LIPTON, Sampson (edited by). Persistent Pain: Modern Methods of Treatment. Vol. 1. Pp.ix+272. ISBN-0-12-451701-3. (London: Academic Press; New York: Grune and Stratton, 1977.) £10.50; \$20.50.
- MEEK, G. A., and ELDER, H. V. (edited by). Analytical and Quantitative Methods in Microscopy. (Society for Experimental Biology Seminar Series, Vol. 3.) Pp.276. ISBN-0-521-21404-1. (Cambridge, London and New York: Cambridge University Press, 1977.) Hard cover £12; Paperback £4.75.
- NEUWIRT, J., and POKKA, P. Regulation of Haemoglobin Synthesis. Pp.206. ISBN-90-247-1999-2. (The Hague: Martinus Nijhoff, Medical Division, 1977.) Dfl.50.
- ROSE, A. H. (edited by). Alcoholic Beverages. (Economic Microbiology, Vol. 1.) Pp.xiv+760. ISBN-0-12-596550-8. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £25.
- SABINE, John R. Cholesterol. Pp.xii+489. ISBN-0-8247-6516-8. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr.82.
- SIEGEL, Malcolm R., and SISLER, Hugh D. (edited by). Antifungal Compounds. Vol. 1: Discovery, Development, and Uses. Pp.xvi+600. ISBN-0-8247-6557-5. (New York and Basel: Marcel Dekker, Inc., 1977.) SFr.175.
- TARG, Russell and PUTHOFF, Harold. Mind-Research: Scientists Look at Psychic Ability. (Introduction by Margaret Mead and Foreword by Richard Bach.) Pp.xxv+230. ISBN-0-244-01424-2. (London: Jonathan Cape, 1977.) £4.95.
- WATER PURIFICATION IN THE EEC: a State-of-the-Art Review. (Water Research Centre.) Pp.vii+467. ISBN-0-08-021225-5. (Oxford and New York: Pergamon Press, 1977. Published for the Commission of the European Communities.) £21.
- WOLFE, Douglas A. (edited by). Fate and Effects of Petroleum Hydrocarbons in Marine Ecosystems and Organisms. (Proceedings of a Symposium, November 10-12, 1976, Olympic Hotel, Seattle, Washington.) Pp.xix+478. ISBN-0-08-021613-7. (Oxford and New York: Pergamon Press, 1977.) £22.25.

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- EYSENCK, Michael W. *Human Memory: Theory, Research and Individual Differences*. (International Series in Experimental Psychology.) Pp.x+366. ISBN-0-08-020405-8. (Oxford and New York: Pergamon Press, 1977.) £8.
- FOREYT, John Paul. *Behavioural Treatments of Aesity*. (Pergamon General Psychology Series, Vol. 61.) Pp.xiv+511. ISBN-0-08-019902-X. (Oxford and New York: Pergamon Press, 1977.) £7.50.
- LABOV, William, and FANSHIEL, David. *Therapeutic Discourse: Psychotherapy as Conversation*. Pp.x+392. ISBN-0-12-432050-3. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) \$15; £10.65.
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- RACHMAN, S. (edited by). *Contributions to Medical Psychology*, Vol. 1. Pp.viii+243. ISBN-0-08-020511-9 (V. 1). (Oxford and New York: Pergamon Press, 1977.) £8.

Sociology

- BLACKSELL, Mark. *Post-War Europe: A Political Geography*. Pp.205. ISBN-0-7129-0789-0. (Kent: William Dawson & Sons Ltd., 1977.) £6.
- DAY, P. R. *Methods of Learning Communication Skills*. (Social Work Series. Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.vii+329. ISBN-0-08-018953-9. (Oxford and New York: Pergamon Press, 1977.) Hard cover £10.50; Flexi cover £7.50.
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- HALL, Raymond L. (edited by). *Black Separatism and Social Reality: Rhetoric and Reason*. Pp.xvii+280. ISBN-0-08-019509-1. (Oxford and New York: Pergamon Press, 1977.) Hardback £12.50; Flexi cover £6.
- SHANKS, Michael. *European Social Policy, Today and Tomorrow*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xi+105. ISBN-0-08-021444-4. (Oxford and New York: Pergamon Press, 1977.) Hardcover £6.50; Flexi cover £2.75.
- TILBURY, D. E. F. *Casework in Context: a Basis for Practice*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.ix+338. ISBN-0-08-019743-4. (Oxford and New York: Pergamon Press, 1977.) Hardcover £8; Flexi cover £5.

Anthropology

- BEALS, Ralph L., HOIJER, Harry, and BEALS, Alan R. *An Introduction to Anthropology*. Fifth edition. Pp.xvii+748. ISBN-0-02-307450-7. (New York: Macmillan Publishing Co., Inc.; London: Collier Macmillan Publishers, 1977.) £5.85. (Pub. 1 Aug. '77).
- NGUBANE, Harriet. *Body and Mind in Zulu Medicine: An Ethnography of Health and Disease in Nyswa-Zulu Thought and Practice*. (Studies in Anthropology.) Pp.xvi+184. ISBN-0-12-518250-3. (London and New York: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1977.) £6.80; £13.25.
- OAKLEY, Kenneth Page, CAMPBELL, Bernard Grant, and MOLLESON, Thea Ivitsky (edited by). *Catalogue of Fossil Hominids. Part 1: Africa*. Second edition. Pp.210+21 plates. ISBN-0-565-05661-1. (London: British Museum (Natural History), 1977.) £12.

Education

- COLES, Edwin K. Townsend. *Adult Education in Developing Countries*. Second edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xviii+199. ISBN-0-08-021293-X. (Oxford and New York: Pergamon Press, 1977.) £5.
- FARAGO, P. J., FRAZER, M. J., and WALKER, S. D. *Chemical Education in Europe*. Pp.vi+380. ISBN-0-85186-659-X. (London: The Chemical Society, 1976.) £3.50; \$7.
- WARNOCK, Mary. *Schools of Thought*. Pp.176. ISBN-0-571-11161-0. (London: Faber and Faber, Ltd., 1977.) Cloth £5.50; Paper £2.95.

General

- CHEREMISINOFF, Paul N., and MORRESI, Angelo C. *Environmental Assessment and Impact Statement Handbook*. Pp.ix+438. ISBN-0-250-40158-4. (Ann Arbor: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) \$32.45; £19.75.
- CLARK, Kenneth. *Animals and Men: Their Relationship as Reflected in Western Art from Prehistory to the Present Day*. Pp.240 (219 illustrations, 84 in colour). ISBN-0-500-23257-1. (London: Thames and Hudson, Ltd., 1977.) £10.50 net.
- DESMOND, Ray. *Dictionary of British and Irish Botanists and Horticulturists, including Plant Collectors and Botanical Artists. With historical introduction by William T. Stearn*. Pp.xxvi+747. ISBN-0-85066-089-0. (London: Taylor and Francis, Ltd., 1977.) £40 net. (No pub. date).
- FITZGERALD, Ross (edited by). *Human Needs and Politics*. Pp.xvi+278. ISBN-0-08-21402-9. (Rushcutters Bay, NSW, Oxford and New York: Pergamon Press, 1977.) (No pub. date).
- FOX, James J. *Harvest of the Palm: Ecological Change in Eastern Indonesia*. Pp.xv+290. ISBN-0-674-38111-4. (Cambridge, Mass. and London: Harvard University Press, 1977.) £10.25. (No pub. date).
- HEIKOFF, Joseph M. *Coastal Resources Management: Institutions and Programs*. Pp.xiii+287. ISBN-0-250-40157-6. (Ann Arbor: Ann Arbor Science Publishers, Inc.; Chichester: John Wiley and Sons, Ltd., 1977.) \$18.15; £11. (Pub. 10 Aug. '77).
- INTERNATIONAL CONFERENCE ON ELECTRICAL VEHICLE DEVELOPMENT, 31 May-1 June. Pp.104. ISBN-0-901223-63-8. (Organised by the Electric Vehicle Development Group) (Stevenage, Herts: Peter Peregrinus, Ltd., 1977.) UK £6.40; Overseas £7.50 (excl. Americas). (Pub. Aug. '77).

- JOHN, J. A., and QUENOUILLE, M. H. *Experiments: Design and Analysis*. Second edition. Pp.296. ISBN-0-85264-222-9. (London and High Wycombe: Charles Griffin and Company, Ltd., 1977.) £12 net.
- McINTOSH, I. G., and MARSHALL, C. B. *The Face of Scotland*. Third edition. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xiv+230. ISBN-0-08-021320-0. (Oxford and New York: Pergamon Press, 1977.) Hard cover £7; Flexi cover £4.
- NEW METHODS AND TECHNIQUES (Scholarly Publishers Guide). Pp.101. ISBN-0-906083-00-1. (Primary Communications Research Centre, University of Leicester).
- MORSE, Philip M. *In at the Beginnings: a Physicist's Life*. Pp.vii+375. ISBN-0-262-13124-2. (Cambridge, Mass. and London: The MIT Press, 1977.) \$14.95.
- PECCEI, Aurelio. *The Human Quality*. (Pergamon International Library of Science, Technology, Engineering and Social Studies.) Pp.xii+214. ISBN-0-084 021480-0. (Oxford and New York: Pergamon Press, 1977.) Flexi cover £3.25; \$6. Hard cover £6.50; \$12.
- PERSINGER, Michael A., and LAFRENIERE, Gyslain F. *Space-Time Transients and Unusual Events*. Pp.xiii+267. ISBN-0-88229-334-6. (Chicago: Nelson-Hall, Publishers, 1977.) Cloth \$9.95; Paper \$5.95.
- PRZELECKI, Marian, SZANIAWSKI, Klemens, and WOJCICKI, Ryszard. *Formal Methods in the Methodology of Empirical Sciences*. (Proceedings of the Conference, Warsaw, June 17-21, 1974.) (Synthese Library, Vol. 103.) Pp.457. ISBN-90-277-0698-0. (Dordrecht, Holland and Boston, Mass.: D. Reidel Publishing Company; Wrocław, Poland: Ossolineum Publishing Company, 1976.) Dfl. 105; \$39.50.
- RABEN, Joseph (edited by). *Computer-Assisted Research in the Humanities*. (A Directory of Scholars Active.) Pp.251. ISBN-0-08-019870-8. (Oxford and New York: Pergamon Press, 1977.) £27.50.
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- ROZDZIENSKI, Walenty. *Officina Ferrari*. (A Polish poem of 1612 describing the noble craft of ironwork.) Translated by Stefan Pluszczewski. Edited by W. Rozanski and Cyril Stanley Smith. Pp.xvi+123. ISBN-0-262-18079-0. (Cambridge, Mass. and London: The MIT Press, 1976. Published jointly with the Society for the History of Technology.) £9.40.
- RUSSELL, Bertrand. *Roads to Freedom*. Pp.158. ISBN-0-04-335033-X. (London: George Allen and Unwin (Publishers), Ltd., 1977. First published 1918.) £1.50 net.
- RUSSELL, Bertrand. *Education and the Social Order*. Pp.158. ISBN-0-04-370080-2. (London: George Allen and Unwin, Ltd., 1977. First published 1932.) £1.50 net.
- RUSSELL, Bertrand. *Sceptical Essays*. Pp.189. ISBN-0-04-104003-1. (London: George Allen and Unwin (Publishers), Ltd., 1977. First published 1935.) £1.50 net.
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- RUSSELL, Bertrand. *Political Ideals*. Pp.80. ISBN-0-04-320120-2. (London: George Allen and Unwin (Publishers), Ltd., 1977.) First published in UK 1963.) £1.25 net.
- RUSSELL, Bertrand. *ABC of Relativity*. Third revised edition. Edited by Felix Pirani. Pp.160. ISBN-0-04-521002-0. (London: George Allen and Unwin (Publishers), Ltd., 1977. First published 1925.) £1.50 net.
- Scientific Glassblowing and Laboratory Supplied Catalogue. Pp.124. (Sawbridge-worth, Hertfordshire: R. B. Radley and Co., Ltd., 1977.)
- SCIENCE YEAR: The World Book Science Annual, 1977. (A Review of Science and Technology During the 1976 School Year.) Pp.432. ISBN-0-7166-0577-5. (Chicago and London: Field Enterprises Educational Corporation, a Subsidiary of Field Enterprises, Inc., 1977.) £6.25.
- SCHUMACHER, E. F., *A Guide for the Perplexed*. Pp.155. ISBN-0-244-01496-X. (London: Jonathan Cape; 1977.) £3.95.
- SELL, R. C., CRAWLEY, J. E., CROCKFORD, G. W., and FOX, J. G. (contributions by). *Human Factors in Work, Design and Production*. Pp.xviii+138. ISBN-0-85066-076-9. (London: Taylor and Francis, Ltd., 1977.) £7.
- SEN, Buddhadev. *To Secure "Certain Unalienable Rights"*. Pp.xiv+203. (Baton Rouge, Louisiana: B. Sen, Louisiana State University and Agricultural and Mechanical College, 1977.) \$7.50.
- SHAFER, Wade H. (edited by). *Masters Theses in the Pure and Applied Sciences Accepted by Colleges and Universities of the United States and Canada*, Vol. 20. (A Publication of the Center for Information and Numerical Data Analysis and Synthesis, CINDAS.) Pp.xv+291. ISBN-0-306-34120-4. (New York and London: Plenum Press, 1976.) \$30.
- SITCHIN, Zecharia. *The Twelfth Planet*. Pp.384. ISBN-0-04-113001-4. (London, Boston, Mass. and Sydney: George Allen and Unwin, 1977.) £5.50.
- TOMKIES, Mike. *Alone in the Wilderness*. Pp.215. ISBN-0-354-04142-8. (London: Macdonald and Jane's, Ltd., 1977.) £4.95 net.
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- WALLISER, Bernard. *Systèmes et Modèles: Introduction Critique à l'Analyse de Systèmes*. Pp.248. ISBN-2-02-004638-5. (Paris: Editions du Seuil, 1977.) np.
- WILDEN, Anthony. *System and Structure: Essays in Communication and Exchange*. Pp.xxx+540. ISBN-0-422-76210-5. (London: Tavistock Publications, 1977. Distributed in the USA by Harper and Row Publishers, Inc.; Barnes and Noble (Import) Division. First published 1972.) £5.25.
- WHITE, Gilbert. *The Natural History of Selborne*. Edited with an Introduction and Notes by Richard Mabey. (The Penguin English Library.) Pp.xviii+283. ISBN-0-14-043-112-8. (Harmondsworth, Middx.: Penguin Books, Ltd., 1977. First published 1788-9.) 95p; USA and Canada \$2.95.
- WORLD ARMAMENTS AND DISARMAMENT: SIPRI Yearbook 1977. Pp.xvi+421. ISBN-0-262-19160-1. (Stockholm: Stockholm International Peace Research Institute, in collaboration with Almqvist and Wiksell International, Stockholm and The MIT Press, Cambridge, Mass. and London, 1977.) Sw.kr. 140.
- VON WEIZSACKER, C. F. *The History of Nature*. Pp.vi+191. ISBN-0-226-89189-5. (Chicago and London: The University of Chicago Press, 1977.) £4.50.
- YOURGRAU, Wolfgang, and BRECK, Allen D. (edited by). *Cosmology, History, and Theology*. Pp.xvi+416. ISBN-0-306-30940-8. (New York and London: Plenum Press, 1977.) \$54.
- ZIMAN, John. *The Force of Knowledge: The Scientific Dimension of Society*. Pp.ix+374. ISBN-0-521-09917-X. (Cambridge and London: Cambridge University Press, 1977.) £4.75.

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announcements

On the Move

Dr E. B. Carstens, Department of Microbiology, Centre Hospitalier Universitaire, Sherbrooke, Canada, to Institute of Genetics, University of Cologne, Germany.

Details of changes of department, sabbaticals, where leave will be taken and so on should be sent to On the Move; there is no charge for this service.

Appointments

Dr E. H. Francis, to the Chair of Earth Sciences, University of Leeds, from 1 October 1977.

Professor H. Charnock, Director of the Institute of Oceanographic Sciences, to the Chair of Physical Oceanography, University of Southampton, from 1 February 1978.

Dr P. Crutzen, to Director of the Atmospheric Quality Division of the National Center for Atmospheric Research (NCAR).

Professor R. N. Pryor, to President-Elect of the Institution of Mining and Metallurgy from 20 June 1978.

Awards

Dr G. N. Hounsfield has been awarded the Royal Society Mullard Award for 1977 (£1,000) in recognition of his conception and development of the EMI-Scanner, a computerised three-dimensional X-ray system.

Two Technology Writers' Awards, sponsored by ITT Business Systems under the auspices of British science writers, will be awarded for work published between 1 January and 31 December 1977. One will be for work published in professional periodicals, trade and technical magazines, or national and regional newspapers, the other for material broadcast on radio or television. Both prizes will be £1,000 cash and £500 for travel or equipment. All entries are by submission (to J. Anstiss, MPR Ltd, 293 Gray's Inn Road, London WC1, UK), and it is hoped to announce the winners in February 1978.

Marconi International Fellowship

Nominations, to be received by 15 October 1977, are invited for the 1978 Fellowship (\$25,000), established in 1974 to commemorate Guglielmo Marconi's contributions to scientific discovery, engineering and technology. The recipient, in recognition of out-

standing contribution in these fields, is invited to give a public lecture based on the work during the 12 months following presentation. Address all enquiries to The Marconi International Fellowship Council, Aspen Institute for Humanistic Studies, 1919 Fourteenth Street, No. 811, Boulder, Colorado 80302, USA.

National Academy of Sciences Committee on Aerobiology

A new programme has been outlined by a NAS committee to encourage the advancement of Aerobiology as a discrete science. The NAS wish to compile a register of aerobiologists, giving details of research interests. Please send relevant information to National Research Council, Assembly of Life Sciences, 2101 Constitution Avenue, Washington, DC 20418.

Meetings

30 September, **Fine Structure and Chemical Shifts in Electron Spectroscopy**, London, UK (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

30 September–1 October, **Measurement in Biological Electron Microscopy**, Bristol, (Physiology Departmental Office, The Medical School, University of Bristol, Bristol, UK).

2–8 October, **2nd International Colloquium on Physical and Chemical Information Transfer in Regulation of Reproduction and Ageing**, Varna, Bulgaria (Secretariat, Colloquium '77, Central Laboratory of Biophysics, Acad. G. Bonchev Str. Bl. 6, 1113 Sofia, Bulgaria).

3–5 October, **5th Biennial Symposium on Turbulence**, Rolla (Turbulence Symposium, Extension Division, University of Missouri-Rolla, Rolla, Missouri 65401).

3–7 October, **Thermodynamics of Magnetic Fluids**, Udine, Italy (B. Berkovsky, Science Sector, UNESCO, 7 place de Fontenoy, 75700 Paris, France).

5 October, **Technology Transfer from the Nuclear Industry**, Harewell (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London, UK).

7–11 October, **VII International Congress of Essential Oils**, Kyoto (Y. Kato, Secretary General, VII International Congress of Essential Oils, c/o Kyoto International Conference Hall, Takaraike, Sakyo-ku Kyoto, 606 Japan).

10–13 October, **XXV International Meeting on Transportation and Com-**

munications, Genoa (International Institute of Communication, Villa Piaggio, Via Pertinace, 16125 Genova, Italy).

11–13 October, **4th Annual UMR-DNR Conference on Energy**, Rolla (N. Fleming, Conference Coordinator, Extension Division, University of Missouri-Rolla, Rolla, Missouri 65401).

11–13 October, **AGU Conference on Oceanic Fronts**, New Orleans (American Geophysical Union, 1909 K St, NW, Washington, DC 20006).

17–18 October, **Conference on Genetic Epidemiology**, Hawaii (N. E. Morton, School of Public Health, University of Hawaii, Honolulu 96822).

17–21 October, **1st International Congress on Phosphorus Compounds and their Non-fertiliser Uses**, Rabat, Morocco (IMPHOS (World Phosphate Rock Institute), 8 rue de Penthièvre, 75008 Paris, France).

19–21 October, **Symposium on Current Topics in Drug Research**, Uppsala (R. Dahlbom, Biomedical Center, University of Uppsala, Box 574, S-751 23 Uppsala, Sweden).

24–26 October, **ASTM Symposium on Erosion: Prevention and Useful Applications**, Vail, Colo (Miss J. B. Wheeler, ASTM, 1916 Race Street, Philadelphia, Pa. 19103).

24–28 October, **International Conference on Water Chemistry of Nuclear Reactor Systems**, Bournemouth (The Conference Office, Institution of Civil Engineers, 1–7 Great George Street, London SW1, UK).

25–28 October, **International Conference—Radar 77**, London, UK (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

31 October–3 November, **Advancing Energy Technology**, Eastbourne (The Institute of Fuel, 18 Devonshire Street, Portland Place, London W1, UK).

31 October–4 November, **Symposium on Radioimmunoassay, and Related Procedures in Medicine**, Berlin (International Atomic Energy Agency, PO Box 590, A-1011 Vienna, Austria).

31 October–4 November, **Conference on Water Chlorination: Environmental Impact and Health Effects**, Gatlinburg (R. L. Jolley, Chemical Technology Division, Oak Ridge National Laboratory, PO Box X, Oak Ridge, Tennessee 37830).

2–3 November, **International Symposium on Polarography in Action**, London, UK (The Secretary, Scientific Symposia Ltd, 42–43 Gerrard Street, London W1, UK).